ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF
ENVIRONMENTAL HEALTH SCIENCES
FISCAL YEAR 1974

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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ANNUAL REPORT

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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

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OFFICE OF THE DIRECTOR

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Summary Statement

The Fiscal Year 1974 was characterized by involvement in an ever-widening circle of environmental problems with continuing highly limited fiscal and personnel resources. Because of these limitations it was necessary to restrict program development to only two Branches -- Mutagenesis and Biophysics -- and to accomplish this growth required program reductions in other high priority programs. However, even in the face of this continuing growth, the Institute was turned to increasingly to provide consultation and advice regarding an ever-increasing number of environmental problems.

CONSULTATION AND LIAISON

The liaison activities continue to be carried out both on a scientist-to-scientist basis and on an Institutional basis. The Director and members of his staff have served as advisors to the Office of the Secretary, and represent the Department in the consideration of a wide variety of current environmental problems.

The staff of the Institute continues to provide scientific review of a wide variety of criteria and standards documents both for the National Institute for Occupational Safety and Health and the Environmental Protection Agency. The Director formally represents this Institute as observer on the Policy Advisory Board of the National Center for Toxicological Research, which insures exchange of information in the closely related missions of these two organizations. As a principal responsibility, the Director serves as Chairman of the DHEW Committee to Coordinate Toxicology and Related Programs. The Committee was established by the Assistant Secretary for Health. The purpose of this Committee is to serve as an Inter-Departmental group to provide a medium to assure exchange of information on toxicology and related programs, to coordinate these programs, to enhance sharing of resources, and to provide advice to the Department.

Two major interagency activities highlighted the current year. The first of these is the Duluth, Minnesota problem of asbestos in its drinking water supply; the second activity is the health aspects of the energy crisis. In the case of the asbestos problem, the Institute was able to provide technical consultation and the resources of the NIEHS supported Center at Mt. Sinai Hospital, New York, under the direction of Dr. Irving J. Selikoff. In the case of the energy problem, the Institute has been planning programs that can result in data which will clarify the relative risks associated with various energy alternatives. Currently, the Director is serving as a member of an Office of Management and Budget Committee to develop the most appropriate allocation of the energy funds requested in the 1975 President's Budget.

SCIENTIFIC CONFERENCES AND WORKSHOPS

The continuing interest of the scientific community led the Institute to continue its series of conferences and workshops as a means of assessing the current state of knowledge and possible impact on human health. This permits scientists to identify research that should be undertaken or sponsored by participating agencies and organizations. Conferences sponsored during 1974 were:

October 1-2, 1973	Low-Level Lead Toxicity
November 18-20, 1973	Biological Effects of Ingested Asbestos
April 17-19, 1974	Impact of Mobile Emissions Controls
May 9-10, 1974	Heavy Metals in the Environment, Part II
May 14-15, 1974	Biometeorological Consequences of Environmental Controls

These conferences provide the forum for free interchange of communication and face-to-face communication between scientists working on different aspects of the same broad scientific problem. Further, by inviting the participation of the press, these conferences serve a useful means of communicating research findings to the public. In addition, through its journal, Environmental Health Perspectives, the Institute publishes the proceedings of these conferences in order to insure maximum communication of research findings.

INTERNATIONAL PROGRAMS

The Director, his immediate staff, and a number of the Institute's scientists are actively participating in four international agreements:

1. Program of the Joint Committee on Health Cooperation of the United States - Soviet Health Exchange
2. The segment on the Genetic and Biological Effects of Pollutants of the US-USSR Environmental Protection Agreement
3. The section of the bilateral agreement with France (NIH-INSERM) dealing with basic pulmonary reactions to inhaled pollutants
4. The Panel on Methods to Evaluate Environmental Mutagenesis and Carcinogenesis of the US-Japan Cooperative Medical Sciences Program.

In addition, during FY 1974, Dr. Hans L. Falk, Associate Director for Program, has been on detail to the Environmental Health Section of the World Health Organization in Geneva. He has been working to help develop international criteria documents for environmental hazards.

CONTRACTS

AMERICAN ACADEMY OF PEDIATRICS, EVANSTON, ILLINOIS (NO1-ES-2-2093)

TITLE: "Research on Early Detection of Environmental Agents that Induce Diseases in Children"

CONTRACTOR'S PROJECT DIRECTOR: S. L. Harrison, M.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D.

DATE CONTRACT INITIATED: June 1, 1972

CURRENT ANNUAL LEVEL: $52,000

PROJECT DESCRIPTION

OBJECTIVES: The contractor will establish and manage programs at three Medical Centers focusing on the early detection of environmental agents that induce congenital malformations or other diseases in children.

METHODS EMPLOYED: Records are prepared at the three Medical Centers by selected medical students relative to pertinent environmental exposure histories of the child and the parents. These exposure histories, as well as pertinent clinical information, are summarized by the Medical Center Principal Investigator and submitted to the AAP Committee on Environmental Hazards. The Committee reviews the summaries, prepares relevant commentary, and forwards the information to NIEHS as quarterly progress reports.

MAJOR FINDINGS: The Academy arranged the participation of three pediatric centers in clinical epidemiologic efforts. Reports of detailed studies of approximately 200 cases were reviewed and evaluated by the Academy. Study of some 500 obstetric records did not support a cause and effect relationship between progestational agents and congenital heart disease suggested by preliminary study findings.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Detection and identification of environmental stressors continues as an important biomedical research endeavor. New leads for laboratory and epidemiologic investigations are being generated through this system which provides a mechanism for monitoring the fetus and newborn for evidence of deleterious effects due to various environmental influences.

PROPOSED COURSE: This contract will terminate 9/30/74.

BATTELLE NORTHWEST-PACIFIC NORTHWEST LABORATORY, RICHLAND, WASHINGTON
(Interagency Agreement Between NIEHS and AEC, #300A00444)

TITLE: "Study of the Combined Effects of Smoking and Inhalation of Uranium Ore Dust, Radon Daughters and Diesel Oil Exhaust Fumes in Hamsters and Dogs"

CONTRACTOR'S PROJECT DIRECTORS: J. F. Park, Ph.D. and B. O. Stuart, Ph.D.

PROJECT OFFICER (NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: June 1, 1968

CURRENT ANNUAL LEVEL: $325,000

PROJECT DESCRIPTION

OBJECTIVES: As a major part of an integrated research program on the effects of exposure to radon daughters on the development of lung cancer in uranium miners, the following animal studies were initiated:

1. Groups of hamsters were exposed to the following combinations:

   a. radon daughters on room air nuclei,
   b. radon daughters and carnotite ore dust,
   c. diesel engine exhaust alone,
   d. diesel exhaust fumes, radon daughters and ore dust,
   e. carnotite ore dust.

Concentrations of radon daughters, composition of ore dust and concentrations of other toxic agents in the diesel oil exhaust fumes were carefully controlled. After being exposed to predetermined cumulative dosage levels, the animals were maintained for life for the observation of pathological changes, particularly lung cancer and emphysema.

2. To test specifically the possible synergistic effects of radon daughters and cigarette smoking, studies were initiated on three groups of 18 dogs as described below:

   a. dogs exposed to radon daughters and carnotite ore dust exposure for four hours per day, 7 days per week at concentrations of 15 nanocuries per liter; with sham smoking;
   b. dogs exposed to radon daughters and ore as in (a), plus two working shifts of smoking (total of 10 cigarettes per day with resting on alternate hours).

Regular clinical, radiological and radiographic examinations will be made during and after the smoking and radon exposures. The dogs, except for one in each group which will be sacrificed for early histopathological studies, will be held for their life span with observations for pulmonary changes and lung cancer as the end point.

MAJOR FINDINGS: The hamster studies were completed during FY 74.

Sixty beagle dogs continue to receive daily inhalation exposures to cigarette smoke and/or 600 WL of radon daughters with uranium ore dust. These animals have received 50 months of exposures to date. An acute respiratory syndrome, similar to the one occuring in dogs exposed to high levels of plutoniom-239 has necessitated the sacrifice of seven dogs exposed to radon daughters plus dust and six dogs exposed to radon daughters, dust and smoking. It is to be expected that the remaining original dogs in these two groups will require sacrifice in the near future. The histopathological findings for the dogs that have died or were sacrificed have been described in detail in several Battelle progress reports.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These experiments were designed to determine, in both a short-lived animal, the hamster (life-span 2-3 years), and a long-lived species, the dog (life-span 12-13 years), the possible synergistic effects of chemical agents--dust, cigarette smoking and diesel exhaust fumes--operating singly and in concert with exposure to radon daughters upon the production of lung cancer and other lung diseases in animals. To the extent possible in the laboratory the exposure conditions are designed to simulate those to which uranium miners have been exposed, and in whom an increased incidence of lung cancer has been observed.

PROPOSED COURSE: NIEHS support for the dog studies will not continue beyond FY 74.

BATTELLE PACIFIC NORTHWEST LABORATORIES - RICHLAND, WASHINGTON (NIH-72-2043)

TITLE: "Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $89,700

PROJECT DESCRIPTION

OBJECTIVES: To determine, using advanced radiotracer, spectroscopic and chromatographic techniques: (1) the rate and extent of uptake of metals from soils by representative grass and broad leafed plants, (2) the translocation of metals in plants, (3) the effect of metals on the soil microbiota and soil microbial process, (4) the nature of metal bond types and chemical forms of metal metabolites in plant and microbial tissues, and (5) the potential for the soil formation of organometal complexes, subsequent uptake of these compounds by plants and entrance into the human food chain; inherent in these objectives is the need to develop suitable methods for determining the chemical forms of metals, and their fate in soils, plants and microbial tissues.

METHODS EMPLOYED: Ritzville soil was thoroughly mixed with labeled and unlabeled isotopes of arsenic, chromium, mercury, thallium and tungsten. A split-root (soil/nutrient solution) method was employed to grow soybeans to maturity in the metal containing soils. The plants were cultured in a growth chamber under constant light (14 hr light,; 10 hr dark), temperature (27°C light; 20°C dark) and humidity (40-45%). At maturity the plant tops and roots were harvested (133 to 142 days from planting). Plants were separated into cotyledons, roots, stems, leaves, immature bean pods, mature bean pods and mature beans. In all cases the tissues were analyzed for total metal radioisotopes.

With regard to the microbial studies, major emphasis was placed on the characterization of those organisms that exhibited high resistance to metals using various enrichment techniques at different metal concentrations. In the initial studies viable counts were used as a measure of growth, in the more recent work turbidimetric and dry weight measurements have been used.

MAJOR FINDINGS: The use of a split-root system allowed the investigator to grow the soybean plants to maturity in the constant temperature growth chamber; studies have been completed for the metals arsenic (As), chromium (Cr), mercury (Hg), thallium (Tl) and tungsten (W). The split-root system of soybean culture resulted in a marked increase in yield compared to the pot culture technique. The increased growth had a marked effect on the distribution of metal in the mature plants. However regardless of the method employed, the order of Tl concentration in mature tissues was stems → leaves → beans. The yields of tissue and uptake and distribution of metals of plants grown on soils containing added As, Gr, Hg, Tl and W using the split-root method were not significantly different, although

yields of plants exposed to As were generally reduced.

Chromium is accumulated in markedly higher concentrations in the root and cotyledon tissues than the other plant structures. Mercury showed no significant accumulation in any of the plant tissues. Thallium in contrast to Gr was not concentrated in the root tissue. Tungsten was accumulated mainly in the leaves; the measurements indicated that W was uniformly distributed in the leaves and not concentrated in the leaf veins as in the case of thallium. Of all the metals studied, W was present in highest quantities in the mature seeds.

The purpose of the microbial studies has been to determine the mechanism by which soil microbiota surviva and reproduce in the presence of heavy metals. During the past year, work has concentrated on the effect of thallium concentration on the growth of resistant isolates. Aerobic bacteria were markedly resistant to the effects of thallium. The aerobic spore-forming bacteria were markedly resistant to thallium concentrations of 30 μg/ml or greater. In general, fungal isolates showed marked resistant to thallium.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides, desiccants or as contaminants of fertilizers. The importance of soil and plants in this pathway has been underscored by the spectre of metal conversion to more soluble and more toxic heavy metal complexes which may subsequently be taken up by plants and thereby enter directly into human foodstuffs. Particular attention should be given to determining the mechanisms for converting inorganic metals to organic complexes and the extent of this conversion. This information should provide a sound basis for estimating the potential hazard arising from the continued use of these materials and may provide the key to remedial measures.

PROPOSED COURSE: The split-root technique will be used to grow soybean plants to maturity utilizing Ag, Cd, Sb and Ni. In the microbial area, three resistant organisms of those analyzed to date will be utilized to study microbial conversions of thallium. This is primarily to determine if microbial conversion of the labeled metal is occupying and the form of the metal made by the microorganisms will be compared to that found in the soil mixture in an effort to determine the mechanism of resistance.

BROOKHAVEN NATIONAL LABORATORY - UPTON, NEW YORK
(Interagency Agreement)

TITLE: "Chemical and Environmental Mutagen Studies Utilizing the Tradescantia Test System"

CONTRACTOR'S PROJECT DIRECTOR: A. H. Sparrow, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED: June 6, 1972

CURRENT ANNUAL LEVEL: $168,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of the proposed research are: (a) to demonstrate the usefulness of and further develop the Tradescantia somatic mutation test system as a quantitative monitor in environments containing known or suspected mutagens; (b) to use special genetic clones of Tradescantia to establish the relative mutagenicity of a limited number of mutagens (primarily in gaseous form), as to magnitude of effect, specificity of action, and possibility of reversion; (c) to look for evidence of genetic repair of DNA injury produced by chemical mutagens and to look for possible threshold levels below which induced mutation rate is negligible; and (d) to determine in greater detail the time and dose response for several loci for one selected gaseous mutagen, comparing these with presently available radiation data and with results observed in animal cells.

METHODS EMPLOYED: Two different clones of Tradescantia (clone 02 and clone 4430), which are heterozygous for a flower petal color mutation were used to determine the mutagenicity of exposure to various concentrations of SO_2, NO_2 and ozone. Refinements in chemical mutagen exposure techniques were made to establish 1,3-dibromoethanol (DBE) as the mutagen standard in these studies rather than ethylmethane sulfonate (EMS). Plant breeding and new clone selection has also been continued to obtain more sensitive clones which might provide better indicators of weak mutagens and chronic levels of exposure. New clones with a wider range of spontaneous mutation rates at each of 3 loci are being used along with existing mutable clones to develop methods for studying repair.

MAJOR FINDINGS: Exposures to ozone and SO_2 showed that these gases had only a very slight mutagenic effect in stamen hairs after 5 or 6 hour exposures to 0.5, 1.0, 2.0, and 4.0 ppm SO_2 or 1.0 and 2.0 ppm ozone. At higher concentrations of 50 or 100 ppm SO_2 or NO_2 for 6 hours, a small but significant increase in the mutation rate was found in stamen hairs. Ozone exposures of 6 hours duration in the range of 10-100 ppm showed a slight increase in the mutation rate in stamen hairs or about twice the control rate in 3 of 4 of the exposures. Exposures have also been made to N_2O for 6 hours ranging in concentration from 50 to 500 ppm; scoring of this experiment is still in progress.

Technical problems with the use of EMS as a standard have forced the investigators to seek another mutagen to use as a standard and exploritory work is pro-

gressing using DBE. Both mutagens give equally high mutation rates with the Tradescantia system.

Experiments with mutable clone 6 (radiation-induced in clone 02) which was thought to be repair deficient was shown to be more radiation sensitive than clone 02 after x-irradiation and possibly (in preliminary data) after exposure to EMS.

Data on somatic mutation rates in new heterozygous clones and Tradescantia species has shown a wide range of sensitivity in both radiosensitivity and spontaneous mutation rates.

New facilities have been constructed to permit more precise exposure of Tradescantia to various cases which permits more accurate control of gas concentration and flow rate.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract should provide a badly needed assay for mutagenic airborne environmental chemicals. It is extremely sensitive to ionizing radiation and the exploratory work with selected chemical mutagens during this past year suggests that it may be able to detect concentrations as low as 10 ppm. This assay system should provide a meaningful assay for the production of gene mutations and relates to the Institute's program to develop sensitive assay systems to detect the mutagenic activity of environmental chemicals.

PROPOSED COURSE: At the conclusion of the present course of oxidant exposures, work will focus on determining the relative mutagenicity of specified industrial compounds using DBE as the standard. DBE will also be used as a standard to determine the relative chemical mutagen sensitivity of several other clones of Tradescantia for comparison with known sensitivity to radiation. Reasons for the comparable radiation sensitivity of clones 02 and 4430 to ionizing radiation and about an 8-fold difference in sensitivity between these two clones to DBE and EMS are as yet unknown.

THE DOW CHEMICAL COMPANY, MIDLAND, MICHIGAN (NIH-NIEHS-73-2102)

TITLE: "Studies of the Effects of Environmental Components on Reproduction and Ontogenesis"

CONTRACTOR'S PROJECT DIRECTOR: B. A. Schwetz, D.V.M., Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: February 5, 1973

CURRENT ANNUAL LEVEL: $275,267

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the teratogenic potential in animals of environmental components when administered singly or in combination, (2) to determine species differences in response to teratogens and to study the etiology of such differences and (3) to investigate the mechanism of action of teratogens and to improve experimental testing and extrapolation to man.

METHODS EMPLOYED: In general, the screening approach outlined by the World Health Organization (Technical Report No. 364, 1967) is being followed with certain modifications to alleviate some of the problems encountered by private industry during the testing of potential therapeutic agents.

MAJOR FINDINGS: Dichlorvos, a widely used aerosol insecticide, was selected for study, both as a single agent and in combination with other agents. This was an effort to demonstrate potentiation of developmental toxicity.

Experimentation revealed that the mouse could tolerate orally adminstered dichlorvos at doses up to 60 mg/kg/day but that the rabbit could tolerate only 5 mg/kg/day. At these dose levels, fetal malformations were not detected in either species, although an increase in the incidence of resorptions was noted among the treated rabbits.

Alcohol was tolerated in the drinking water of both species at a concentration of 15%. Positive teratogenic effects did not result when females were exposed by way of their drinking water to alcohol during organogenesis, nor were such effects detected when both alcohol and dichlorvos were administered simultaneously to pregnant mice.

Similarly, carbon monoxide (250 ppm) was not teratogenic in either species after administration by inhalation for 7 hr per day during organogenesis. Carboxymethemoglobin levels attained were 11 to 13%. Effect of oral administration of dichlorvos with simultaneous inhalation of 250 ppm of carbon monoxide in air is being studied.

SKF-525A, an inhibitor of the hepatic mixed function oxidase system, was not teratogenic in mice after intraperitoneal injections during organogenesis at a dose level of 35 mg/kg/day. However, simultaneous administration of SKF-525A

and dichlorvos resulted in increased toxicity in the pregnant mouse. Moreover, following such a co-administration of SKF-525A and dichlorvos, neither with apparent teratogenic potential, a significant increase in cleft palate was observed. Further studies are necessary to identify the teratogen.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The etiology of about 60% of human congenital malformations is currently unknown. It is likely that chemical and physical factors in man's environment, acting either singly or in combination, might account for an important fraction of human birth defects.

This contract attempts to carefully evaluate the teratogenic potential of selected environmental factors either alone or in various combinations. In addition, attempts will be made to define basic teratogenic mechanisms which will allow for better predictive extrapolation from experimental animals to man.

PROPOSED COURSE: Dichlorvos is to be administered by inhalation to determine whether this route alters its toxicity in the mouse or rabbit. The SKF-525A and dichlorvos interaction in mice will be studied further. Other potential environmental teratogens will be tested in the future, and those which are positive will be studied further in combination studies.

DUKE UNIVERSITY, DURHAM, NORTH CAROLINA (NO1-ES-3-2117)

TITLE: "Development of Microwave Instrumentation"

CONTRACTOR'S PROJECT DIRECTOR: William T. Joines, Ph.D.

PROJECT OFFICER (NIEHS): Robert H. Wyatt, Ph.D.

DATE CONTRACT INITIATED: June 1, 1973

CURRENT ANNUAL LEVEL: $15,267.00

PROJECT DESCRIPTION

OBJECTIVES: The proposed work of this contract is threefold: (1) Study the electrical dipole model of the neural membrane and its implications, and to determine the unknown parameters in the interaction of the dipole model with microwave radiation, (2) Develop microwave instrumentation and methods to investigate microwave-neuron interactions over the 0.2 to 3.0 GHz frequency band, (3) Develop the instrumentation and dosimetry of a more accurate method of determining the microwave power absorbed by a neuron.

MAJOR FINDINGS: Using computer oriented skull models, radiation dosage levels within the skull were investigated for a range of microwave frequencies. With 10 mW/cm^2 of incident radiation at 2.1 GHz, the power density reaches a maximum of about 20 mW/cm^3 near the center of the brain for a model of 7 cm radius. These calculated power density levels were used as a dosage guide in irradiating live neurons positioned inside a strip transmission line. It was also shown that the homogeneous sphere model of the skull is in error between 1 and 3 GHz; thus this model should not be used to determine potential hazards over this frequency range.

Two equivalent circuits for modeling microwave-neuron interactions were investigated. For the perfect rectifier equivalent circuit with 10 mW/cm^2 incident radiation, the induced transmembrane potential is 27.5 volts, 10,000 times larger than the voltage necessary to influence neuron functioning under the employed experimental set up. For the dipole model equivalent circuit, under certain assumptions, depolarization of the neural membrane can exceed the threshold value necessary to fire the neuron. Sample calculations predict that microwave pulses 8 to 80 microseconds wide, applied at a rate less than about 100 pulses per second, should be most effective for nerve stimulation. The theory predicts a minimum power level, and perhaps a maximum power level for optimum microwave-neuron interaction. These predictions are shown to be consistent with results of other investigators.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract work on isolated cells and organs will complement the present work in progress at NIEHS, where experiments on the neurological effects of microwaves are being conducted. Work at NIEHS concerns effects in mammalian systems, while the Duke work concerns effects in isolated ganglia of cold blooded animals.

PROPOSED COURSE: This contract, being a "pilot" type contract will terminate in September of 1974. Until this date, the objectives will remain unchanged.

PUBLICATIONS

Joines, W.T., Spiegel, R.J.: A semiclassical theory for nerve excitation by a low intensity electromagnetic field. Bulletin of Mathematical Biology, vol. 35, Oct. 1973.

Joines, W.T. and Spiegel, R.J.: Resonance absorption of microwaves by the human skull, IEEE Trans. on Biomedical Engineering, Vo. BME-21, Jan. 1974.

GULF SOUTH RESEARCH INSTITUTE, NEW ORLEANS, LOUISIANA (NIH-NIEHS-72-2083)

TITLE: "Interaction of Metallic Salts In Vitro: Cell Biological, Analytical and Virological Aspects"

CONTRACTOR's PROJECT DIRECTOR: B. H. Sweet, Ph.D.

PROJECT OFFICER (NIEHS): J. W. Spalding, Ph.D.

DATE CONTRACT INITIATED: April 10, 1972

CURRENT ANNUAL LEVEL: $75,060

PROJECT DESCRIPTION

OBJECTIVES: To explore the interaction of important environmental trace metals in their effect on mammalian cells in tissue culture including their response to viral infection. This toxic effect has been described for certain trace metals in specific ratios and specific situations. A more general evaluation of the situation is desirable.

MAJOR FINDINGS: Experimental protocols for studying acute and chronic heavy metal toxicity on WI-38 cells in vitro were devised. Parameters for determining and comparing degrees of toxicity were established. Due to limited time, only a preliminary study on metal interaction in vitro was undertaken. The use of flameless atomic absorption to measure the metal ion uptake by cells met with difficulty and only late in the study did the method achieve reliability.

Of the five heavy metals extensively studied, Ni, Cd, and Cu were consistently more toxic than Zn or Mn. In acute toxicity studies, actively growing WI-38 cultures were more sensitive to the metals than were stationary cultures. No toxic effect was observed however when actively growing cells were chronically treated for nine passages (nine weeks) with sub-threshold levels of Cd, Ni, or Zn. Chronic toxicity studies on stationary cultures were variable, but treatment with Ni resulted in enhanced aneuploidy and the appearance of chromosomal aberrations. Preliminary studies on metal interaction in vitro indicated that Zn could protect against Cd toxicity; Ni appeared to enhance Cd toxicity.

Cd, Zn, and Ni uptake by WI-38 cultures was measured by flameless atomic absorption. Cd uptake by cells was as high as 16%; Ni and Zn uptake was much lower, 2-3%, and 0.6-2%, respectively. Zn did not appreciably interfere with Cd uptake, so the protective action of Zn cannot be linked to the prevention of Cd uptake by the cells.in this system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The human exposure to various trace metals is generally accepted to be a multiple metal event leaving a fair amount of speculation regarding the identify of the responsible metal in the etiology of the disease. It is also known that a protective interaction is frequently observed when combined exposure occurs. A better understanding of these complex biological systems and interactions is necessary. This contract attempted to explore such interaction--synergistic

or antagonistic--of trace metals on human cells in tissue culture to serve ultimately as a model for studies in the intact animal.

PROPOSED COURSE: This contract expired August 1, 1973.

INSTITUTE FOR MEDICAL RESEARCH - CAMDEN, NEW JERSEY (NIEHS-73-2114)

TITLE: "Somatic Mutations Induced by Biological and Chemical Agents"

CONTRACTOR'S PROJECT DIRECTOR: W. W. Nichols, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED:

CURRENT ANNUAL LEVEL: $49,069

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this work has been to determine whether virus infection in mammalian cells can produce gene mutations at specific loci.

METHODS EMPLOYED: Mutation rates in two different human Lesch-Nyhan cell lines (LN 284 and GM 177) have been determined at the HGPRT locus. A comparison has been made between control cell lines and those transformed with SV40 (LNSV).

MAJOR FINDINGS: SV40-transformed cells were shown to be T-antigen positive as measured by the indirect fluorescent assay, whereas nontransformed cells showed no fluorescence. Effects to select revertants in control and SV40-transformed LN 284 cell lines yielded no revertants in either control or EMS-treated cells.

SV40-transformed and control cell lines of GM 177 cells were also used in reversion experiments at the HGPRT locus. About thirty times as many apparent revertant clones were found in the uninfected GM 177 cells than the SV40-transformed cells showing a striking effect of virus infection of the spontaneous mutability of the HGPRT locus.

A comparison of extracts of LN 284 and extracts of GM 177 with antibody to human HGPRT in immunodiffusion studies showed precipitin lines identical to normal human cell lines. This assay shows that the HGPRT locus is present in both cell lines and that the difference in mutability must be due to some other genetic difference.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to the efforts of the Institute to identify specific mutagenic hazards of environmental importance to man. A number of viruses including those contained in certain vaccines have been demonstrated to produce chromosome damage in mammalian cells. It is important to know whether heritable gene mutations are also produced. The significance of the program concerns the necessity of reducing the mutagenic risk to future generations through the identification of specific hazards.

PROPOSED COURSE: This contract was terminated on April 8, 1974. The contractor proposes to continue this work on funds which he expects to be made available through a grant application to NIH.

THE JACKSON LABORATORY - BAR HARBOR, MAINE (NIH-NO1-ES-4-2159)

TITLE: "Development of a New Mouse Strain to Maximize the Sensitivity of a Point Mutation Assay"

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): Lawrence R. Valcovic, Ph.D.

DATE CONTRACT INITIATED: February 19, 1974

CURRENT ANNUAL LEVEL: $33,845

PROJECT DESCRIPTION

OBJECTIVES: The sensitivity of a point mutational assay system is directly related to the number of loci that can be tested in each individual. In the present system in our laboratory nine loci are sampled. Other inbred strains of mice possess enzyme variants which can possibly be incorporated into one of our present strains. This contract seeks to fully identify these additional variants (at least 13) and then by repeated back crossing to incorporate them into the C57BL/6J genome.

METHODS EMPLOYED: Starch gel electrophoresis is used to determine whether a strain carries an allele that is not found in either the DBA/2J or C57BL/6J strains. When such a variant is found, the strain is back crossed repeatedly to C57BL/6J for 8-10 generations to develop a new strain which will be homozygous with C57BL/6J. Electrophoresis tests will be performed on progeny in each generation to insure that the allelic differences already present in the C57BL/6J line are maintained.

MAJOR FINDINGS: The particular strains carrying 13 additional variants have been identified and initial crosses of these to the C57BL/6J line have been initiated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: While many compounds in the environment have been shown to cause point mutations in lower organisms, fewer compounds have been tested in mammals due to the lack of a feasible test system. Also, a program to monitor the human population for the detection of electrophoretic mobility mutations is in the developmental stages. However, no comparable study has been conducted with any laboratory mammal and hence, there is no base-line data for known mutagens for this type of mutational event. In an attempt to elucidate these areas, we are presently using two mouse strains that have nine different isozymes which can be detected electrophoretically. Since maximal sensitivity depends on polymorphic loci, it is desirable to increase the number of enzyme differences between the two strains. There are at least 13 other allelic differences which are present in various other strains or stocks of mice. If these were incorporated into one of our strains, this would greatly increase the sensitivity of the system with a minimal increase in efforts. The strain will be made available for mutagenesis testing of environmental pollutants, carcinogens and drugs to the scientists in the Environmental Mutagenesis Branch.

PROPOSED COURSE: The offspring obtained from crosses will be analyzed for particular loci and those carrying the desired alleles will be back crossed to C57BL/6J. The procedure will be carried for 8-10 generations.

THE JACKSON LABORATORY - BAR HARBOR, MAINE (NIH-NO1-ES-4-2156)

TITLE: "Development of a System for Detecting Lethal Mutations in Mice"

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): William Sheridan, Ph.D.

DATE CONTRACT INITIATED: January 15, 1974

CURRENT ANNUAL LEVEL: $50,000

PROJECT DESCRIPTION

OBJECTIVES: At present no efficient test system exists for detection of point mutations in mammals. The aim of the contract is to develop strains of mice, carrying marked inversions, which may be used in studies of induction of point mutations. Theoretically these tests will be comparable to the system presently used in Drosophila. The prime goal is to induce inversions in as many chromosomes of the mouse as possible and combine them in a number of strains to be used as tester stocks.

METHODS EMPLOYED: The contractor intends to induce chromosome breaks and inversions by means of radiation and/or chemical agents. He will mate the treated animals and in the F_1 make cytological analysis of one testis of each individual male. If a significant increase in anaphase bridges appears, this is taken as an indication that the animal may carry an inversion, and he is mated to get offspring for further study. Following cytological and linkage studies to characterize the aberration, decision is made as to the utility of the inversion and appropriate marker genes are introduced into the stock.

MAJOR FINDINGS: The effective date of this contract is January 15, 1974. However, prior studies by the contractor had illustrated the ability to induce inversions which could be utilized in point mutation detection.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recessive lethal mutations are known to exist at high frequencies in human populations. Many environmental compounds are known to be mutagenic and capable of causing both chromosome aberrations and point mutations. With an efficient animal model to study the induction of such mutation, more accurate estimates can be made of the risks such compounds imply to the human population.

PROPOSED COURSE: The estimated length of the contract is five years.

KRESGE HEARING INSTITUTE - UNIVERSITY OF MICHIGAN, ANN ARBOR, MICHIGAN
(NO1-ES-2110)

TITLE: "Interaction of Noise and Ototoxic Drugs on Hearing Loss in Animals"

CONTRACTOR'S PROJECT DIRECTORS: Merle Lawrence, Ph.D. and Joseph Hawkins, Ph.D.

PROJECT OFFICER (NIEHS): Reginald O. Cook

ASSISTANT PROJECT MONITOR: Philip W. Albro, Ph.D.

DATE CONTRACT INITIATED: June 15, 1972

CURRENT ANNUAL LEVEL: $100,000

PROJECT DESCRIPTION

OBJECTIVES : To determine the extent and the nature of the interaction between noise and ototoxic drugs in experimental animals, and to study the biochemical mechanisms involved in such losses. More particularly, objectives are:

1. Prove or disprove the involvement of phospholipid metabolism in the destruction of inner ear tissue and function under the influence of neomycin.

2. Investigate the biochemical changes induced by noise exposure (pO_2, ATP, Na^+/K^+, protein and lipid metabolism).

3. Investigate the biochemical changes under the influence of a combined noise and drug treatment. Monitor and correlate ATPase as a function of time after treatment.

4. Determine the mechanism of action of neomycin on polyphosphoinositide metabolism.

MAJOR FINDINGS: It has been determined that when nerve ending preparations of guinea pig cortex are incubated with ^{32}P, ^{32}P is incorporated into the following phospholipids - phosphatidylinositolphosphate, phosphatidylinositol-diphosphate, phosphatidic acid and phosphatidylinositol. Concentrations of 10^{-4} M kanamycin and neomycin and 10^{-3} M of streptomycin maximally increase labelling of the above phospholipids from ^{32}P in guinea pig cortex homogenate. Maximal stimulation of ^{32}P incorporation was 300% of controls for phosphatidylinositolphosphate. These concentrations are similar to serum concentrations of 20-50 μg/ml attained after clinical use of the antibiotics. Sucrose density gradient fractionation of homogenates indicated that this effect was most localized in nerve-ending fractions (synaptosomes).

10^{-4} M ethacrynic acid lowered ^{32}P-labelling of all lipids by approximately 70%, probably due to uncoupling of oxidative phosphorylation.

Methods have been worked out by which successful incorporation of labelled phosphate into the phospholipids of the cochlea can be achieved. Perfusion is through the round window and allows "Pulse" labelling. Differences in

labelling amongst the various phospholipids following treatment with drug were noted.

Electrophysiological results show the time course of neomycin effect dramatically ranging from no change at 3 days to nearly complete impairment after 35 days. Treatment with drug after noise exposure produced potentation of the effect, but little change was noted in pre-noise exposure drug treatment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The burden from excessive noise in the general population is extensive and even more so among industrial workers. It now appears that certain drugs may greatly potentiate this noise damage at low levels of noise. This contract proposes to delineate suspected synergisms and investigate the biochemical mechanisms by which such damage occurs.

PROPOSED COURSE: No change from that specified in contract renewal document, December 15, 1973.

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
ALBUQUERQUE, NEW MEXICO
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Respiratory Tract Deposition Models"

CONTRACTOR'S PROJECT DIRECTOR: Otto G. Raabe, Ph.D.

PROJECT OFFICER (NIEHS): PHillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: November 11, 1971

CURRENT ANNUAL LEVEL: $75,000

PROJECT DESCRIPTION

OBJECTIVES: The major objectives of this research are:

(1) Construction of physical models of the respiratory tracts of man and several animal species;

(2) Measurement of the deposition of aerosols as small as 0.01 micron diameter in the models;

(3) Determination of deposition of selected particle sizes in animals and in man if practical;

(4) Development of a theoretical model of deposition in the lung based on the above results;

(5) Field evaluation of the physical-theoretical model.

MAJOR FINDINGS: Progress has been made on objectives 1-4. Morphometric measurements on lung casts of man and several animal species are complete. Physical models of the respiratory tracts are being constructed. The determination of deposition of 5 particle sizes of 2 physical densities in animals has begun.

The morphometric measurements have classified branches of tracheobronchial trees according to length, diameter, branching angle, angle to gravity and any unusual characteristics. Each branch is numbered according to a novel ordering system that can be used to relocate and re-measure any particular branch region. An algorithum especially suited to tree-like models has been used to generate a computer probabilistic model of the lung based upon the ordered morphometric data. A computer calculation of deposition in this model can be accomplished using any improved mathematical formulation describing the experimental deposition data being accumulated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Improved respiratory tract deposition models are needed to develop insight into the manner in which the comparative morphology of the lung influences the deposition of inhaled particles in experimental animals and man. In particular, such a model would aid in establishing quantitative dose-response relationships

laboratory experiments and would effect more meaningtul extrapolations to human exposure situations.

PUBLICATIONS

Phalen, R. F., Yeh, H. C., Raabe, D. G. and Velasquez, D. J.: Casting the lungs in situ. The Anatomical Record. 177: No. 2, 255-263, 1973.

MIDWEST RESEARCH INSTITUTE, KANSAS CITY, MISSOURI (NIH-NIEHS-72-2090)

TITLE: "An Appraisal of Environmental Exposure to Heavy Metals"

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D.

DATE CONTRACT INITIATED: April 17, 1972

CURRENT ANNUAL LEVEL: $53,000

PROJECT DESCRIPTION

OBJECTIVES: To explore environmental contamination with specific metals which may be hazardous to health. To search the literature and other reports and to seek expert opinions from industry in order to compile these data on potential hazards of specific metals. Special emphasis is placed on determination of the physical parameters and chemical properties, valency, reactivity, etc., as the metal compound makes contact with man. The information is to be put together in a document on each of the metals of specific interest.

MAJOR FINDINGS: The first document dealt with the metal osmium. It contained data on the reactivity of osmium compounds in the environment. The search for information itself was very informative considering that a lot of information is being gathered by many scientists in many laboratories, but with regard to toxic trace metals the nature of the compound or complex is usually not determined which is a function of the level of the metal in the environment and the lack of specificity of the analytical method used.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals or different forms of metals, or their complexes. The information will determine whether research on the health effects of the compound in question should be undertaken and in which direction it should go.

PROPOSED COURSE: The information is being gathered for a number of metals of importance from the toxicologic viewpoint. Reports on palladium and silver are forthcoming. These are to be followed by reports on selenium, tellurium, and zirconium.

MIDWEST RESEARCH LABORATORIES, KANSAS CITY, MISSOURI (NIEHS-72-2084)

TITLE: "Long-Range Toxicologic Effect of Metal-Containing Dithiocarbamate Fungicides"

CONTRACTOR'S PROJECT DIRECTOR: C. C. Lee, Ph.D.

PROJECT OFFICER (NIEHS): R. L. Dixon, Ph.D.

DATE CONTRACT INITIATED: February 1, 1972

CURRENT ANNUAL LEVEL: $138,775

PROJECT DESCRIPTION

OBJECTIVES: The overall objective of this research program is to evaluate the potential toxicologic effects of selected metal-containing dithiocarbamate fungicides, especially their major degradation products. The specific objectives include: (1) To investigate the stability of ferbam, maneb, and zineb in commercial formulations upon ordinary storage conditions and/or seeds of crops and/or growing plants, long-term toxicologic studies will be initiated for these four compounds, the parents, and the major degradation products. (2) To study the rate of absorption, retention, storage, blood levels, excretion, and metabolic product of the selected compounds. (3) To elucidate the toxic effects on the fertility and reproductive performance in male and female rats, to determine the teratological effects in rats and mice, and define the perinatal and postnatal effects in rats of the selected compounds. (4) To determine the acute, subacute, and chronic toxicities of the selected compounds.

METHODS EMPLOYED: Conventional chromatographic procedures, U.V., visible, I.R., spectroscopy, NMR and mass spectrometry, histological techniques, and radiotracer analyses.

MAJOR FINDINGS: During the past year, the following studies were completed. Chemical analysis of bedding material for phenolic compounds and animal feed for the presence of chlorinated pesticides and trace metals, stability of ferbam and thiram on green and dried bean seedlings, stability of thiram alone or in animal feed, fertility and reproductive performance studies of ferbam and thiram in male and female rats, teratological effects of ferbam and thiram in rats and mice, effect of ferbam and thiram on perinatal and postnatal development subacute toxicity of thiram in rats, and cytotoxicity of ferbam and thiram in human culture cells. Studies of the distribution and metabolism of ferbam-^{35}S including the excretion in bile, passage across placenta and secretion to milk, and chronic toxicity of ferbam in rats are being continued. In addition, studies on the metabolism and distribution of ferbam-^{14}C and chronic toxicity of thiram in rats have been initiated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These investigations will establish the effect and nature of the acute, subacute, and chronic toxicities of the selected environmental chemicals and their most

significant degradation products. This information will serve to form a rational basis for evaluation of the potential health hazards produced by the use and exposure of these chemicals.

PROPOSED COURSE: To continue the specified objectives of the contract in relationship to the metabolism and the potential toxic effects of various environmental chemicals. This contract provides the NIEHS with a "quick response" capability to study environmental chemicals that are thought to be potentially hazardous to human health.

MILES LABORATORY - ELKHART, INDIANA (NIEHS-73-2113)

TITLE: "Genetics Tests to Characterize Specific Locus Mutants in Neurospora crassa"

CONTRACTOR'S PROJECT DIRECTOR: L. T. Chang, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED:

CURRENT ANNUAL LEVEL: $54,425

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this contract is the genetic characterization of presumptive adenine-3 (ad-3) mutants of Neurospora crassa derived from experiments at the Institute from forward-mutation experiments with various environmental chemicals as well as various chemical carcinogens.

METHODS EMPLOYED: Stock cultures of presumptive ad-3 mutants have been characterized by a series of heterokaryon tests with various standard tester sets which determine the genotypes of the mutants, complementation pattern of ad-3B mutants showing allelic complementation and also distinguishes point mutations from chromosome deletions. During the latter part of FY 74, the contract was expanded to include making mutants homokaryotic and to prepare silica gel stock cultures.

MAJOR FINDINGS: During the past year tests on mutants from a variety of experiments have been successfully completed. These include heterokaryon tests on mutants induced in haploid strains, predominantly ultraviolet induced mutants in six different UV-sensitive strains as well as the standard wild-type strain. In addition mutants from a wide variety of experiments with a two-component heterokaryon were also completed. Mutagens used in treatment were (1) various carcinogens including epoxides, naphthylamines, 4NQO and 4HAQO, (2) x-rays (10 r/min and 1000 r/min) with exposures predominantly in the range of 500 r to 5000 r, and (3) hycanthone.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to various programs in this Institute where it is essential to characterize the mutants induced at specific loci in this organism with regard to the type of lesion (e.g. point mutation or chromosomal deletion as well as the nature of the damage to the DNA at the molecular level). This is essential in our program to determine the correlation between carcinogenic and mutagenic activity as well as to determine whether chemical carcinogens characteristically produce any particular type of lesion at the molecular level. In addition, the characterization of the specific locus mutants produced by environmental chemicals enables us to better determine their relative hazard for man.

PROPOSED COURSE: During FY 75, the majority of the mutants analyzed will come from experiments with (1) carcinogen induced mutants in excision-repair deficient strains, (2) carcinogen induced mutants in standard wild-type strains, (3) struc-

tural analogs of hycanthone and lucanthone (IA-3, IA-4, IA-5, IA-6) as well as other antischistosomal agents, and (4) radiation-induced mutants in radiation-sensitive strains.

ENVIRONMENTAL INFORMATION SYSTEM OFFICE
OAK RIDGE NATIONAL LABORATORY - OAK RIDGE, TENNESSEE
(Interagency Agreement Between NIEHS and ORNL)

TITLE: "Environmental Mutagen Information Center"

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTE: National Cancer Institute

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: $133,250

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and disseminate chemical mutagenesis information.

MAJOR ACCOMPLISHMENTS: EMIC's data file at the end of March 1974 contained 12,000 bibliographic entries. Of these, approximately 11,000 have been indexed with respect to agent(s), organism(s), and Chemical Abstract Service Registry Number(s). The Center processes requests for information at a rate of 1-2 per day. These requests came primarily from governmental agencies in the United States or government supported research in universities. Several requests from other countries are also received.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators, and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (1,250). EMIC has helped several national commissions in collecting and extracting the data from the literature. For the Institute and especially the Environmental Mutagenesis Branch, EMIC saves more than one man-year of time for the personnel in the Branch in supplying the literature references in special areas.

PROPOSED COURSE: EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objects as well as indexes for agents and selected title keywords. As great a portion as possible of EMIC's total bibliographic entries will be keyworded with respect to agent, test organism, and test object. EMIC will continue to answer selected requests for special information from the scientific, educational, and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in interest.

PUBLICATIONS

Wassom, John S. and Takenka, Yusuke: Activities of the Environmental Mutagen Information Center. Review Journal of the National Institute of Hygienic Sciences (Japan) Vol. 7(1): 1-17, 1974.

Wassom, John S.: The literature of chemical mutagenesis. Chapter 34 in Chemical Mutagens, Vol. III, Alexander Hollaender (Ed.), Plenum Press, pp. 271-287, 1973.

Wassom, John S. and Malling, H. V.: Mutagenic activity of environmental chemicals. (Abstract of a paper delivered at a symposium on "The Potential Genetic Effects of Environmental Pollutants on Man" held February 18-March 1, 1974 in Moscow.

Wassom, John S.: The Environmental Mutagen Information Center (EMIC). Mutation Research 21: 242-243, 1973.

Wassom, John S.: The Environmental Mutagen Information Center (EMIC) and carcinogenesis research. Abstract of a paper delivered to the Second Annual Collaborative Carcinogenesis Conference, San Antonio, Texas, November 25-29. 1973, Carcinogenesis Program Proceedings, pp. 137.

Huff, J. E. and Wassom, J. S.: Health hazards from chemical impurities: chlorinated dibenzodioxins and chlorinated dibenzofurans. Intern. J. Environ. Studies, Vol. 6 (in press).

von Halle, E. S., Generoso, W. M. and EMIC Staff. Chemical mutagenesis in mammals. III. A bibliography on the effects of chemicals on mammalian germ cells or germ cell tissue. ORNL-EMIC-5, 1973.

EMIC Staff: A survey of the 1972 literature on chemical mutagenesis, ORNL-EMIC-3, 1974.

EMIC Staff: A review of the host-mediated assay system. ORNL-EMIC-4, 1974.

Huff, James Edward and Wassom, John S.: Chlorinated dibenzodioxins and dibenzofurans - an annotated literature collection. Environmental Health Perspectives Exp. Issue No. 5, September 1973, pp. 283-312.

Huff, J. E. and Wassom, J. S.: Hazardous contaminants: chlorinated dibenzodioxins and chlorinated dibenzofurans. Proceedings of the Second Annual Conference on Environmental Chemicals: Human and Animal Health, held at the Colorado State University, Fort Collins, Colorado, July 24-27, 1973.

Huff, James Edward and Wassom, John S.: Safety philosophy in the laboratory. (Abstract of a paper delivered to the First International Conference on Environmental Mutagens, Asilomar, Pacific Grove, California, August 29-September 1, 1973). Published Mutation Research 21 (6): 346, 1973.

SOUTHWEST RESEARCH INSTITUTE, SAN ANTONIO, TEXAS (NIH-72-2035)

TITLE: "The Interaction of Specific Air Pollutants with Lung Lipids"

CONTRACTOR'S PROJECT DIRECTOR: R. Bollinger, Ph.D.

PROJECT OFFICER (NIEHS): Phillip W. Albro, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $161,577

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the effects and mechanisms of interaction of specified gaseous compounds with lung membrane lipids *in vivo* and *in vitro*; to clarify the chemistry and biochemistry of such interactions; to attempt to correlate the structural changes observed on a chemical level with any significant functional changes; and to examine possible reversal, protective or preventative approaches.

METHODS EMPLOYED: The basic test animal is the rabbit; *in vitro* systems include lung slices, lung mitochondria and cells from lung washes. Various conventional chromatographic procedures, NMR and IR spectroscopy and histological techniques are used. ESR is used to detect alterations in membrane components *in situ*.

MAJOR FINDINGS: (1) *In vitro* exposure of rabbit lung to 1 ppm SO_2 resulted in a greatly reduced level of fatty acid β-oxidation and, apparently, a reduced level of activation of fatty acids for lipid synthesis. However, protective mechanisms appear to prevent this effect *in vivo*. (2) Various freons differ in their tendency to inhibit or activate lipase activity in the lung; those Freons that activate lipases also appear to uncouple oxidative phosphorylation. (3) N_2O also appears to activate lipase in the lung, while NO_2 inhibits it.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project studies in detail a specialized aspect of the general chemical responsivity of the lung to commonly inhaled substances, and thus should ultimately aid in predicting responsivity to other substances of interest.

PROPOSED COURSE The final year's work will be devoted to (1) estimating the biological significance of the previous year's findings. (2) Comparing the ESR methods with the enzymatic studies as to utility as a general approach to screening air pollutants for subtle effects on the lung. (3) Evaluating the role of lung lipases in maintaining the functional integrety of the lung.

UNIVERSITY OF MINNESOTA, DULUTH, MINNESOTA (NO1-ES-4-2149)

TITLE: "Collection and Storage of Biologic Specimens Exposed to Orally Ingested Asbestos Fibers"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Carter, M.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D.

DATE CONTRACT INITIATED: November 5, 1973

CURRENT ANNUAL LEVEL: $71,000

PROJECT DESCRIPTION

OBJECTIVES: The contractor will conduct a broad biologic sampling from human and non-human sources exposed to oral ingestion of asbestos fibers.

METHODS EMPLOYED: Autopsy and surgical biopsy tissues will be obtained and stored to ensure usefulness for current and future analysis. Appropriate radiographic materials will be collected and analyzed. Epidemiologic information pertinent to specimen and radiographic samplings will be obtained.

MAJOR FINDINGS: Findings will not be reported by the contractor until six months of the study are completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will produce asbestos tissue burden information basic to determination of the health hazard potential of such exposure. Such studies are an integral part of NIEHS efforts to determine the magnitude and significance of hazards inherent in long-term, low-level exposures to various environmental agents.

PROPOSED COURSE: This contract will be terminated in FY 75.

UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE, ROCHESTER, NEW YORK
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Uranium Mining - Health Hazards Associated with Radon Daughters"

CONTRACTOR'S PROJECT DIRECTOR: William F. Bale, M.D.

PROJECT OFFICER (NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITITATED: June 30, 1968

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: In five separate but closely interrelated projects, senior scientists have conducted the following studies:

Project A - Dr. Donald Morken has exposed dogs and rats to radon and radon daughters on room air nuclei. The magnitude and intensity of exposure versus early histopathological effects are important initially.

Project B-1 - Dr. John Hursh has studied the bone and tissue distribution of ^{210}Pb as well as excretion of ^{210}Pb and ^{210}Po using dogs exposed in Project A.

Project B-2 - Dr. Hursh has measured the transport time of ^{212}Pb from human lungs.

Project C - Dr. Thomas Mercer has characterized the radon daughter carrying aerosols used in the studies by Morken and Hursh.

Project D - Dr. R. W. Helmkamp and Dr. W. F. Bale have developed new techniques for the measurement of ^{210}Po and ^{210}Pb in urine.

MAJOR FINDINGS: All projects have been completed. Publications based on this work are listed below. A final report is due by July 1974.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is an integral part of research directed toward the resolution of the uranium miner-lung cancer problem. This project should yield useful information on induction of lung cancer and on heavy metal metabolism.

PROPOSED COURSE: This work has been completed.

PUBLICATIONS

Hursh, J. B. and Mercer, T. T.: Measurement of ^{212}Pb loss rate from human lungs. Journal of Applied Physiology 28:268-274, 1970.

Mercer, T. T.: The deposition of unattached decay product atoms in an impaction stage. Health Physics 17:259, 1969.

Blair, H. A.: Dose-time relations for induction of lung cancer in uranium miners. Reprint from symposium on "Radiation-Induced Cancer." International Atomic Energy Agency, Vienna (1969).

Hursh, J. B.: Retention of ^{210}Pb in beagle dogs. Health Physics. (In Press).

Helmkamp, R. W., Hrynyszyn, V., Contreras, M., Bale, W. R.: Development of a method for measuring low levels of ^{210}Po in human urine. (In Press).

THE UNIVERSITY OF TEXAS AT AUSTIN - AUSTIN, TEXAS (NIH-NIEHS-70-2288)

TITLE: "Detection of Human Somatic Mutation"

CONTRACTOR'S PROJECT DIRECTOR: H. Eldon Sutton, Ph.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

DATE CONTRACT INITIATED: June 30, 1970

CURRENT ANNUAL LEVEL: $32,444

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this investigation during 1973 was to determine if an elution method for fetal hemoglobin could be used as a somatic cell mutation system for human populations. This exploratory work was also intended to show inherent difficulties in somatic cell systems.

METHODS EMPLOYED: Modifications of the Bethe-Kleihauer technique for detection of fetal hemoglobin containing cells was used. The effect of different parameters such as pH were also investigated and variance among blood samples from normal volunteers was examined.

MAJOR FINDINGS: It has been decided that a wide variety of factors other than mutation can cause a general rise in fetal hemoglobin levels as measured by the Bethe-Kleihauer technique. It was also noted that the test is reliable and repeatable only over a very narrow range of pH.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings demonstrate some of the specifications that a somatic cell mutation system envision and meet. The variant must not occur without mutation; it must be a positive variant and it must be a technique usable by most technicians.

PROPOSED COURSE: This contract was terminated in December 1973.

UNIVERSITY OF WASHINGTON - SEATTLE, WASHINGTON (NIH-NO1-ES-4-2151)

TITLE: "Detection of Point Mutations in Somatic Cells"

CONTRACTOR'S PROJECT DIRECTOR: Peter E. Nute, Ph.D.

PROJECT OFFICER (NIEHS): Christopher N. Frantz, M.D.

DATE CONTRACT INITIATED: January 1, 1974

CURRENT ANNUAL LEVEL: $57,122

PROJECT DESCRIPTION

OBJECTIVES: It is likely that an increase in the mutation rate in the human population would be detrimental, yet there is no system developed to monitor the human population for point mutations. The purpose of this investigation is to develop a simple system to measure point mutations in readily accessible human somatic cells. It is likely that the somatic cell mutation rate would reflect the germ cell mutation rate, and the rate of introduction of genetic defects into the human population.

METHODS EMPLOYED: Many human red cells contain small amounts (less than 1%) of the two types of fetal hemoglobin. The two types differ by one amino acid, and are called a_γ and g_γ. However, rare individuals have genetic disorders of hemoglobin production in which there is only one type of fetal hemoglobin produced. Study of these and other individuals with related genetic hemoglobin production disorders have shown that having only one type of fetal hemoglobin is most likely the result of a deletion of the gene for the other type. The same mutation is expected to occur in individual red blood cells within many humans, though very rarely. The research seeks to produce antibodies specific to each of the two types of fetal hemoglobin, using antigen blood from the rare individuals having only a_γ or g_γ. The two antibodies will be conjugated with different colored fluorescent dyes, and red blood cells from normal subjects will be exposed to the two antisera. Any cells containing only one fetal hemoglobin type will be identified by only one antibody and will fluoresce in only one color, while normal fetal hemoglobin containing red blood cells will fluoresce in both colors.

MAJOR FINDINGS: One hundred normal subjects will be tested for somatic cell mutation rate by this technique. At present, attempts are underway to make the two specific antisera.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system able to detect deletions in the genome of readily accessible somatic cells would allow estimation of the human population rate. It would allow investigation of individuals and populations exposed to a mutagenic risk. There is no simple technique for screening at present. The antisera would also be useful in investigation of thalassemia and related genetic hemoglobin disorders.

PROPOSED COURSE: When the technique is available, it will be used to investigate somatic cell mutation rates with respect to age, and to investigate populations at mutagenic risk such as patients receiving cancer chemotherapy and those exposed to radiation. Screening programs would be able to identify populations exposed to mutagenic substances.

THE UNIVERSITY OF WISCONSIN - MADISON, WISCONSIN (NIH-NIEHS-72-2095)

TITLE: "Mutation Studies with Cultured Human Cells"

CONTRACTOR'S PROJECT DIRECTOR: Robert DeMars, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED: June 15, 1972

CURRENT ANNUAL LEVEL: $86,059

PROJECT DESCRIPTION

OBJECTIVES: Develop a mutational assay system which will provide a means of monitoring mutations among somatic cells of humans. This system shall utilize the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus in diploid, human fibroblasts.

METHODS EMPLOYED: The contractor is expected to isolate mutants at the azaguanine phosphoribosyltransferase (AGPRT)locus by looking for mutants resistant to 8-azaguanine. This work is also to entail a study of both spontaneous and induced forward-mutation rates at this locus.

MAJOR FINDINGS: Two additional fluctuation tests have been performed to estimate the spontaneous rate to azaguanine resistance in human fibroblasts. The true hereditary nature of the azaguanine selected variants has now been further documented by additional demonstrations that clones initially selected out in azaguanine remain resistant after a many fold increase in the absence of the selective agent. Thus, these experiments show that the mutations are stable changes behaving like gene mutations in germ cells and like stable changes that could cause cancer in somatic cells. A large number of spontaneous and chemically induced mutants have been newly isolated and partially characterized in the last year. The great majority of them have significant partial or total reductions in HGPRT activity. The genetic characterization of the variants requires somatic cell hybridization in which the investigator is attempting to detect sites of changes that are not in the structural locus for HGPRT on the X chromosome. The most important single advance has been the production of antibodies directed against purified human HGPRT and AGPRT. These reagents allow him to identify the species specificity of these enzymes in somatic cell hybrids. This is of major importance because the reappearance of HGPRT in hybrids of azaguanine resistant human cells and HGPRT deficient mouse cells is the primary observation that can indicate the mutation in the human cell had occurred at a site other than the structural locus for HGPRT.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to our attempts to develop assays to screen environmental chemicals for mutagenic activity (e.g., production of gene mutations). The development of generalized methodology will make it possible to study spontaneous and induced forward-mutation at the HGPRT locus in any individual in the population. Individuals of portions of the population at high risk can also be examined to determine whether the "spontaneous" frequency of HGPRT

mutants is unusually high. Given cell lines can also be used to screen chemicals for mutagenic activity. Humans heterozygous at the AGPRT locus can usually be identified by pedigree analysis, and fibroblast cultures from these individuals can be used for analysis at either locus.

PROPOSED COURSE: The genetic analysis of azaguanine resistance and diaminopurine resistance and other stable phenotypic changes in somatic cells will require several years. In addition, experiments will be performed to (1) attempt to detect the induction of azaguanine resistant mutations in human fibroblasts by hycanthone and two of its chemical relatives, (2) to perform fluctuation tests with the cell strains used in the mutagenesis experiment, (3) to continue as possible genetic analysis of azaguanine resistance. The basic procedure is to fuse mutant human cells with HGPRT and AGPRT deficient mouse cells and to make the following observations: (a) if HGPRT reappears in the hybrids, is it human? (b) if HGPRT in hybrids is human, is it normal or abnormal? (c) if human HGPRT reappears, must the specific human autosome disappear from the hybrids first?

U.S. FORESTRY SERVICE, RESEARCH TRIANGLE PARK, NORTH CAROLINA (NIEHS-72-2206)

TITLE: "Influence of Microorganisms and Other Soil Factors on Degradation of Certain Insecticides"

CONTRACTOR'S PROJECT DIRECTOR: Felton L. Hastings, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: August 24, 1971, terminated August 24, 1973 and extended 6 months without additional funds.

CURRENT ANNUAL LEVEL: $25,714.00

PROJECT DESCRIPTION

OBJECTIVES: To study the influence of microorganisms and other soil factors on the degradation of certain insecticides, the insecticides of particular interest being Mirex, Kepone, DDVP, Disulfoton, Temik and Dursban.

METHODS EMPLOYED: Special extraction procedures were developed for each of the insecticides studied. Gas and thin-layer chromatography were used in the analysis of the extracts, and radiotracer techniques were used to detect metabolism and to locate the metabolites.

MAJOR FINDINGS: The first year's work was concentrated on Mirex, the current work was concentrated on Temik. No breakdown of Mirex was detected after 6 months in nine aerobic or in four anerobic soils. Further, degradation of Mirex was not detected after 2-week incubations with thirteen individual bacterial species in synthetic liquid culture. In addition, Mirex was shown to have little or no effect on soil microorganisms, even when relatively high concentrations of the compound were added to the soil. Leaching studies have shown Mirex to remain in the soil indefinitely.

Temik was shown to be metabolized primarily to the sulfoxide with lesser amounts of the sulfone, oxime sulfoxide, oxime sulfone, nitrile sulfoxide, and nitrile sulfone also being produced. After 7 days incubation time the ratios of parent compound and metabolites are as follows: Temik 55%, sulfoxide 43%, and other metabolites 2%.

PROPOSED COURSE: The contract has been terminated.

PUBLICATIONS

Stevens, A., and Hodges, C.: Persistence of Mirex and its effect on soil microorganisms. J. Ag. Food Chem., in press, 1974.

OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM

OFFICE OF ASSOCIATE DIRECTOR FOR PROGRAM
Summary Statement

During fiscal year 1973, the Office of the Associate Director for Program initiated a technological surveillance activity with the help of two chemical engineers who became available through the National Science Foundation as part of the program of the President's Internship in Science and Engineering.

Accomplishments by the technological surveillance program are defined as identification of direct or potential health hazards from chemicals in the environment, and human exposure to them. Exposure may be from manufacture, use patterns, or waste disposal practices. All programs are ongoing in nature and involve the gathering of available literature information on the chemical technology of a substance and biological reactions to these substances. This information is then synthesized into a form that either a direct assessment of a potential environmental health hazard can be made, or an identification is made of additional research needs required to make this assessment.

The first stage of this program was to classify the chemical process industries by major product types, i.e., plastics, agricultural chemicals, rubber products, etc. Efforts have been directed at characterizing the products and use patterns of existing industries with an attempt to forecast what chemicals will be developed for future markets. By drawing attention to the dynamics of the chemical process industries and their efforts to remain viable, it forces biologically-oriented scientists engaged in health research to focus on this real-world process which by attempting to solve its technological and economic problems has unwittingly caused many environmental health problems.

Current and ongoing programs involve additives for plastics such as plasticizers, stabilizers, flame retardants, fillers, antioxidants, etc., which are not chemically bonded to the plastic matrix; gasoline additives, alternate additives, emission control devices, and alternate power supplies; antibacterial products; aerosol products; rubber tire compounds and rubber tire dust; alternate energy supplies such as coal gasification and the development of superconductors; and the development of new commercial products such as liquid crystals.

Another aspect of the group's activities is to identify developing chemicals, their projected uses, questions pertinent to the types and routes of human exposure, and the initiation of research required to evaluate toxicity of a substance in its use pattern. This process is being carried out with a new series of chemicals which is in the research and development stage. The chemical series contains a functional group which makes it very attractive for applications as a surfactant and as a component in cosmetics. There is no information available about biological reactions to either the functional group or its series members. Initial Institute efforts are concerned with product

purity and characterization of impurities. This is to be followed by pharmacologic testing of an as yet unspecified nature. This program is unique in that it is developing biological information before a substance is commercialized and the initial efforts are in chemical analysis of product purity before biological testing begins. This program will not only benefit this Institute and its mission, but will also serve as a guide to the Environmental Protection Agency's Office of Toxic Substances which will have the responsibility of assessing toxicity of chemicals for the commercial markets.

To supplement the industrial and environmental surveillance activity, a contract has been supported which searches the literature, obtains reports and makes contact with experts inside and outside industry for information on the chemical and physical properties of metallic compounds reaching man's environment in air, water, food or consumer products which may prove hazardous to man. The information obtained will determine whether research on the health effects of the compounds in question should be undertaken and in which direction it should go.

Because of the need for new antibacterial compounds to replace hexachlorophene or mercury containing products, a search for toxic properties of the proposed replacements brought out evidence that many of the available compounds show some adverse effects.

In a separate activity, the Associate Director was asked by the Director of the Division of Environmental Health, World Health Organization (WHO), to consult and collaborate in the production of a document: Environmental Health Criteria and Standards, which could be the basis of a collaborative effort to obtain excellent information on criteria and serve each country in promulgating their own standards. Following the acceptance of the documents, the preparation of Outlines for Criteria Development was undertaken by WHO and contributions were made by the Associate Director in several areas.

Because of the importance of this activity not only for WHO and the collaborating agencies in other countries, but specifically for the NIEHS in its effort to communicate its findings and obtain information from other laboratories on health problems due to environmental contamination and pollution, the Associate Director was detailed to Geneva during fiscal year 1974 to collaborate on the implementation of the effort and to streamline the contributions of information by the participating agencies in a dozen countries.

Serial No.: NIEHS-ADP-001
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "An Investigation of Interaction of Environmental Pollutants in Causing Porphyria"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Hans L. Falk, Ph.D.

OTHER INVESTIGATORS: Sandra Jordan

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	0.1
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the porphyrogenic properties of many toxic environmental chemicals to obtain a better understanding of chemical structural requirements, indication of mode of action, type of toxicity and prediction of interaction of chemicals producing more or less toxicity relating to porphyria.

METHODS EMPLOYED: Oral intubation of the young and mature rat with the chemical under study. Other methods could involve the developing chick embryo or liver cells in tissue culture. The progress of the metabolic disorder is studied by fluorescence and quantitated by absorption spectroscopy.

MAJOR FINDINGS: Hexachlorobenzene is one of the most effective porphyrogens in man's environment. It will cause long lasting porphyria if administered to rats in the diet. Once the animal is off that porphyrogenic diet, the disorder may not disappear at all or it may decline slowly. Porphyria will be enhanced by a second feeding. Single administration of hexachlorobenzene by stomach tube will lead to porphyria in a few days. Lead acetate given the same way will also cause porphyria in a few days, but this effect is quickly lost after a single administration. The excretion product is coproporphyrin. No uroporphyrin is found. This is in contrast to the elimination of porphyrins after hexachlorobenzene, where both are present in certain relationship to each other.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The variety of chemicals that have been described as porphyrogenic suggests that

either many mechanisms may be at work producing the effect or structural requirements for affecting a particular site work through multiple steps. The chemicals either belong to the group of hypnotic drugs possessing certain chemical requirements which bring them close to industrial pollutants, or to certain complex chlorinated hydrocarbons, such as hexachlorobenzene, the chlorinated dibenzodioxins and furans, the PCB's and a few others. They may also be mycotoxins of which several have been identified, although structural correction is not easy. In a different context they may belong to the group of heavy metals which produce similar effects except by a different pathway. The combination of environmental exposure to the two types of chemicals is very common and although in many cases only marginal may lead to significant metabolic disturbance, particularly when other factors such as dietary ones are not adequately met.

PROPOSED COURSE: Standardize the work on hexachlorobenzene and lead acetate. Evaluate the response to these chemicals in young, normal and old animals. Study the effect of combination of these chemicals with others in producing porphyria. Study the changes in liver on acute or chronic exposure leading to porphyria or its recovery from the disease. Study the structural requirement for porphyrogenesis of the allyl side chain, the acetamide or the ureido group. Search for the environmental appearance of such chemicals of environmental or occupational abundance and determine the activity of breakdown product.

Serial No.: NIEHS-ADP-002
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Technology Surveillance of the Chemical Process Industries"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: Warren T. Piver, Ph.D.

OTHER INVESTIGATORS: Hans L. Falk, Ph.D., Robert E. Munson, Ph.D., and Herbert S. Posner, Ph.D.

COOPERATING UNITS: Institute-wide

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To provide background information about chemicals produced by the chemical industry for the commercial market. By an understanding of material flow from raw material to final product, public health officials and basic scientists charged with protecting the public from exposure to hazardous environmental chemicals, can better plan research to elucidate the mechanism of toxicity before the substance becomes a widespread problem.

METHODS EMPLOYED: Literature review aimed at synthesis of available information on chemical processes, losses of material during processing, production and disposal of by-products, uses of manufactured chemicals, and biological investigations conducted with both the individual chemicals, and the combination or final product formulation. The basic methodology employed has been the classification of the different chemical industries. Then by understanding the products of the existing chemical industries and their associated environmental health problems, some insight into developing industries and future industries can be obtained. In effect, this methodology starts from what is known, and uses it to project what could happen.

Another important method employed in technology surveillance is reading of trade journals and keeping up with developing technology which is in the research and development stage. At this stage, huge monetary outlays have not been made and suggestions for research aimed at elucidation of environmental manifestation of toxicity are more easily received and performed.

Another aspect of this methodology is liaison with other health-oriented Government agencies, particularly the EPA and NIOSH. Coordination with the Office of Fuel Additives and Fuel Additives Registration, EPA, has resulted in the convening of a meeting to discuss the impact of mobile emission control devices and alternative fuel additives to be put into effect to meet the 1976 exhaust emission standards of the Clean Air Act. With NIOSH, attendance at meetings concerned with occupational exposure to vinyl chloride and association of this exposure with hemangiosarcoma of the liver has provided state-of-knowledge about the mechanism of carcinogenesis with this agent and suggested studies to be performed at NIEHS on other important industrial monomers.

MAJOR FINDINGS: Because of the many different types of activities which this office has participated in during this last year, it would be more comprehensible to describe each one briefly.

A considerable effort has been devoted to reviewing EPA Effluent Limitation Guidelines for Point Sources for the Office of the Assistant Secretary for Health, DHEW. Careful consideration was given to the technological feasibility of waste water treatment techniques proposed to meet standards prescribed by the Clean Water Act for effluents discharged from these point sources to receiving waters. Point source categories which have been reviewed were: Textile Mills, Builder Paper and Board Mills, Coal Mine Waste Impounds and Refuse Piles, Inorganic Chemical Industries, Cement Manufacturing, Red Meat and Rendering Processing, Flat Glass, Ferroalloy Smelting and Slag Processing, and Steel Making. Other activities for this office have involved review of Drinking Water Standards, and environmental impact statements.

This office has represented the Institute as a panel member on a NSF Committee on trace contaminants in the environment due to the manufacture and distribution of organic chemicals. The purpose of this Committee is to develop a project description for a contract to be financed by NSF which would rank manufactured organic chemicals by their toxic potential. In this manner, NSF will use this information to set funding priorities for ecological assessment of these chemicals. Membership on this panel includes Government, education, and industrial organizations. This program is similar to NCI's contract with SRI which assists them in setting priorities for their bioassay program of evaluation of carcinogenic potential of chemicals.

With regard to developing technology, considerable attention has been given to the methods proposed to meet the standards of the Clean Air Act, and future energy sources.

Other activities include serving as Project Officer for a Contract with Midwest Research Institute which is providing background information on heavy metals in the environment. This activity has complemented another contract with Battelle Northwest on the fate of heavy metals in soils and plants. In this manner, a better understanding of environmental transport mechanisms for heavy metals can be obtained.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These surveillance and liaison activities will continue to provide necessary information about products and use patterns of chemicals of the CPI, important environmental transport mechanisms, and development of methods to predict exposure levels for environmental chemicals. Such information can be used in planning research programs for the Institute.

PROPOSED COURSE: This work will continue and will expand into other areas of interest. This program will attempt to include more and more Institute personnel and representatives from other disciplines and background.

PUBLICATIONS

Piver, W.T.: Potential dilemma: The methods of meeting exhaust emission standards of the Clean Air Act. Environ. Health Perspec. (in press).

Serial No.: NIEHS-ADP-003
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Health Aspects of Coal Transformation"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Robert E. Munson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS:

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: The production and transformation of coal may be increased and developed along a variety of routes because of the need for (1) extra energy sources and (2) preservation of existing petroleum resources for the isolation of petrochemicals. The estimated fossil fuel reserve of the U.S. is 89% coal. In other parts of the world, oil or gas predominates. The routes for transformation are reviewed as a first step in the evaluation of their health related aspects. Only a few of the considerations can be given.

METHODS EMPLOYED: Literature review.

MAJOR FINDINGS: The gasification of petroleum would use the more scarce U.S. petroleum reserve. Also, while the technology for shale oil production has been thoroughly tested on a laboratory scale development of this energy source has been hindered by cost, potential environmental disruption and land leasing problems. These two, therefore, are not considered here.

Bituminous coal is slightly less than half the U.S. reserve and is found mainly in the East and North Central regions. More than 70% of U.S. coal production is from these fields. This coal burns with little smoke but about 43% contains more than 3% sulfur and about 70% contains more than 1% sulfur. The subbituminous and lignite coals are of lower quality but comprise about 56% of U.S. reserve and are mainly in the Northern Plain and Rocky Mountain states. The reserve is mainly untouched. Both of these contain little sulfur but yield considerable fly ash on combustion.

Anthracite is the highest quality coal but is about 2% of the U.S. reserve.

Processes proposed for coal transformation include: (1) cleaner combustion, (2) solvent refining, (3) pyrolysis, (4) liquefaction and (5) gasification. Clean combustion would result in the removal of sulfur over fluidized limestone giving a low production of sulfur dioxide. In solvent refining the coal is converted into a heavy aromatic solvent, filtered to remove ash and insoluble organics and fractionated. The product contains less than 0.1% ash. All inorganic sulfur and about 65% of the organic sulfur is removed. In pyrolysis gas, oil or tar and a char are produced. These can be used after further treatment. Liquefaction is carried out by catalytic hydrogenation. Sulfur is removed as H_2S which can be converted to other forms of sulfur. Ash is removed physically. The slurry oil and hydrogen can be recycled. Gasification has been proposed above or below ground. The products can include methane, carbon monoxide, carbon dioxide, hydrogen, hydrogen sulfide and sulfur dioxide depending upon the conditions. The gas can be purified to mainly methane. The chemicals to be considered, therefore, include those that contain sulfur, nitrogen, aliphatic or aromatic carbon compounds in the ash or in other products of combustion or transformation, trace metals, catalysts, hydrogen, water in the form of steam and additional chemicals that might be incorporated during transformation. High temperature must also be considered. Some of the advantages and disadvantages of the processes are considered.

There are also the hazards to the miners with strip mining being less hazardous than deep mining. There are potential effects on soil and waterways, some of which might affect human health in the neighboring or even distant areas. These effects are acid mine drainage, erosion, silting of streams, leaching from waste piles and settling of land over mined-out areas. There is the danger of fire or explosion recognized in some situations. A report for NIEHS has been prepared: "The Fuel Shortage and Coal Transmutation", R. E. Munson.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There are potential health hazards at the site of the mining, at the site of transformation of the coal whether this is at the mine or elsewhere and at the site of use of the transformed product. These hazards might derive from the coal, from the chemicals added during transformation or clean burning or from disposal or run-off of the waste products. The hazards will vary with the processes and usages that are developed and it is wise to anticipate and monitor these.

PROPOSED COURSE: The above considerations will be developed further as the course of fossil fuel production and conversion proceeds.

Serial No.: NIEHS-ADP-004
1. Associate Director for Program
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Preventive Surveillance of Environmental Chemicals for Toxic Potential"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Herbert S. Posner, Ph.D.

OTHER INVESTIGATORS: Robert E. Munson, Ph.D. and Warren T. Piver, Ph.D.

COOPERATING UNITS: Environmental Biology and Chemistry and Environmental Toxicology Branches, NIEHS
Ashland Chemical Company

MAN YEARS:

Total:	1.20
Professional:	1.15
Other:	0.05

PROJECT DESCRIPTION

OBJECTIVES: The project develops several modes of preventive surveillance and research appropriate to NIEHS. The program assists the chemical process industries and Federal agencies with a regulatory mission in their attempts to minimize toxic events in general or limited populations. The information that is developed relates first to the compounds being considered and then, as greatly as possible, to related chemical or biologic situations.

METHODS EMPLOYED: Examination of the chemicals for their chemical properties and for properties in the presence of biologic media; consultation with those who have appropriate information; review of the literature; preparation of an evaluation; development of an outline of needed research; discussion with the appropriate groups to perform the research; feedback of the information as it develops; and publication of the results in open scientific literature by the parties concerned to the greatest extent possible.

MAJOR FINDINGS: The first chemicals studied are new to biologic systems and relatively new on the chemical scene. They were first commercially advertised in early 1973 for a wide variety of uses depending upon their structure. The common function of the chemicals is that of an inner salt, $\overset{+}{\geqslant}\text{N-N-}\overset{-}{\text{C}}\text{-O}$, where the CO can be replaced with SO_2 though the latter are not yet offered commercially.

Many different units can occupy the four open positions and thus provide a wide range of properties.

The compounds are called hydrazinium hydroxide inner salts by Chemical Abstracts though they have been referred to alternately. For example, they were reviewed as amine-N-imines in 1972 and this review was translated from German at NIEHS. They are called aminimides by Ashland and were reviewed as such in mid-1973.

It was determined that little or no information exists on: (1) biologic and environmental conversions of the compounds, (2) biologic responses, (3) rates and routes of absorption and excretion, (4) responses of a potentially toxic nature and (5) parameters of persistence in biologic and environmental situations. After visiting with the Company it was agreed that two compounds of their commercial series would be provided in both pilot plant and more pure grades. Dr. Phillip Albro in our Environmental Biology and Chemistry Branch examined the four preparations for the presence of extra components, documented these and purified the more pure of each further. It was learned by communication with Ashland that two pilot plant grades of one of the compounds have been referred to, one being 85-90% pure and the other about 98% pure. We received the 98% sample and it is unclear at present which of these would be offered commercially.

Four reports have been prepared to date for NIEHS: "Potential Hazard: Aminimides" by R. E. Munson (a short initial report); "Aminimides: New Commercial Chemicals" by H. S. Posner; "Aminimides: Proposed Uses and Resultant Routes of Exposure" by R. E. Munson; and "Purity of Two Aminimides" by P. W. Albro and H. S. Posner. Biologic tests are in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Examination of this biologically-new series of chemicals containing two N atoms should provide important data useful for comparative toxicology as well as the information on the safety of the compounds. As such, this is an excellent series of chemicals for NIEHS to be considering as a part of its overall program on the safe use of environmental chemicals.

PROPOSED COURSE: The biologic tests on the aminimides will continue, new chemicals will be considered as these are identified and the methods for surveillance and research will continue to evolve.

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH
Summary Statement

The Environmental Biology and Chemistry Branch is composed of three Sections; Animal Husbandry, Chemistry and Comparative Biology. This organizational framework was developed and adopted during the year to allow the Branch to most effectively meet its obligation in each of these diverse areas of responsibility. The Branch Office currently heads the Chemistry and Comparative Biology Sections.

Major Concerns

A collaborative support program responsive to Institute needs is developing in all areas of Branch responsibility. While these programs are developing in an expeditious fashion, the spectrum of Branch research program as it relates to Institute program remains vague and ill defined. A concise perceptable concept in this area needs to be acknowledged by the Institute. Inattention will likely result in a stagnation of scientific motivation. To develop and manage such an activity, the Branch Office must receive administrative relief through appointment of an individual to head the Chemistry Section.

Considerable thought and effort have gone towards the identification of personnel development patterns that are tangible career ladder opportunities within the Animal Husbandry Section. If capable biological aides are to continue to develop proficiency in performing the more sophisticated tasks associated with the handling and utilization of research animals, "fill in" labor must be provided. For this to occur, Institute practices as to utilization of supplemental labor pools need revision. Without such action, even the current modest efforts cannot endure.

Support Activities

The Animal Husbandry Section expends its total effort in support of intramural research programs. The support activities of the Chemistry and Comparative Biology Sections fluctuate dependent upon the resources required to meet a particular need.

Chemists are frequently consulted within NIEHS so that they may advise scientists in appropriate areas of their research. In a number of instances needed compounds were synthesized or analyzed to determine purity prior to being used by other intramural scientists. Two other projects conducted during the fiscal year are illustrative of the relevance of some of the more sophisticated chemistry support efforts. Both have met with limited success and typify the

long lead time that is inherent in some requests. A variety of approaches were tried in the development of analytical methods to identify and quantify various Aroclors and constituent isomers in biological specimens. One must have a means of relating tissue levels of PCBs to the Aroclor formulation to which a population is exposed if meaningful cause-effect relationships can be correlated with epidemiological data. An NIEHS funded University center is trying to study such a group. In another project, a means to convalently link 2,3,7,8-tetrachlorodibenzo-p-dioxin to serum albumin is being sought. A radioimmunoassay method for TCDD detection would provide a relatively simple means to assess the presence of TCDD in a multitude of environmental milieus. It would also have application to intramural research programs which seek to elucidate the TCDD mechanism of action.

The Comparative Biology Section was able to continue a high level of laboratory support activities even though it lost several personnel. This was accomplished through adopting newer and more efficient methods or through equipment purchases that require less personnel to operate. All histology and EM tissue preparations for the Institute are provided by personnel in this Section. The Hematology Laboratory contributed major portions of its time to the Biophysics group in their study of hematological parameters in several experiments involving Coturnix quail. The Diagnostic Pathology Laboratory continues to provide a major part of the Aerotoxicology Section's histopathology interpretation. The Microbiology Laboratory alerted NIEHS scientists to the possible deleterious research effects of bacterial contamination from deionizer resin beds or contaminated vessels reused for storing distilled water. It has routinely screened many NIEHS tissue cultures for presence of Mycoplasma spp. The Clinical Medicine, Diagnostic Pathology and Microbiology Laboratories have continual major commitments relating to the health and sanitation of sources and facilities associated with the use of research animals.

The quality of performance in the Animal Husbandry Section continued to improve. While the animal population supported per person has started to level off, the number and array of sophisticated techniques regularly performed by the biological aides increases at a steady rate. Indepth expertise in such procedures as intubation, parenteral dosing, inbred colony maintenance, timed matings, artificial breeding, and forced breeding techniques is resident within the Section. Developing these skills in the Section's career employees results in the improved performance of these functions at grade levels more consistent with the requirements of the task. Also implicit in such action is the provision of a career ladder for a major segment of Institute employees.

Several problems continue to challenge the Branch's animal support activities. While rigid and continual health surveillance activities identified reliable sources of healthy animals, the quality of the shipments which arrive at NIEHS are often irrevocably compromised due to difficulties encountered in transportation. The problem is most acute in those animal types with little physiological reserve to adjust to overt changes in environment e.g. inbred mice, rats and pregnant animals. Inproved shipments resulted through contracting by freight airline and abandoning shipments via passenger oriented airlines. Poor reproductive performance in some rodent stocks has impaired several Institute research efforts. Attempts to define the factor(s) associated with this problem have yielded contradictory or at best equivocal results. Feed, water, housing

and infectious agents have been or are being evaluated to determine their respective roles, if any, in the problem. It is likely that in selected instances the solution to the impaired and breeding performance will be through development and maintenance of the appropriate colonies at NIEHS.

Many intrabranch support activities that are common to use of research animals at NIEHS are being reviewed. Thorough re-examination as to current need, methods employed, and utilization of results is being undertaken. Such periodic reviews are necessary to insure that resources be committed to areas that are most critical to Institute animal support requirements. Such redirection is seen by the decreased expenditure of resources for development of the opossum as an animal model and committing these resources to development of protocols for providing several rabbit and mice models for reproductive studies. The logic of such action is evident in the large number of scientists that are dependant upon the latter model; indeed, it is a vital in vivo cog of entire sections or work groups in the Environmental Toxicology and Environmental Mutagenesis Branches.

Research Activities

The Branch is satisfied of the progress made towards developing interrelated research programs within the Branch. The exchange of thoughts and research data in defining and achieving the objectives of a intergrated research project is proving beneficial to scientists in both the Comparative Biology and the Chemistry Sections. Intergration of some of these projects into interbranch efforts needs to be pursued. Modest progress in this regard is in evidence.

In the design and selection of projects, concerted attempts are made to develop objectives which have broad science application while at the same time utilizing chemicals or agents that in themselves are of direct current concern. For example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is utilized in a number of Branch research projects. The exquisitely toxic TCDD is, of itself, a chemical of intense environmental health concern to the chemical industry, the Environmental Protection Agency, USDA, and FDA. Many procedures and protocols promulgated while researching the biological activity of TCDD serve as the basic approach to developing an understanding of the whole class of dioxins, the equally relevant dibenzofurans, as well as other chlorinated halogenated cyclic hydrocarbons. This "multiple pay off" approach needs greater consideration in NIEHS program development if major accomplishments with modest resources are to be realized.

The availability of C^{14} labeled TCDD of relatively high specific radioactivity enabled us to utilize a classical means of determining the chemicals absorption, distribution, secretion, metabolism and excretion in adult, pregnant and nursing animals. Such studies conducted in the rat confirm earlier work which indicated that no significant tissue metabolism of TCDD occurred. The main storage compartments are liver and adipose tissue with early indications that proportional loss of TCDD over time occurs at a faster rate from liver. The presence of metabolites in feces needs further examination relative to source, identity, reabsorption and possible biological effects. Other experiments observed that only modest quantities of TCDD gain access to the fetus with little affinity

for liver storage before the immediate prepartem period. TCDD is readily mobilized from maternal liver and secreted in milk thereby producing neonatal TCDD levels 6-8 multiples higher than the original maternal dose on a μg/Kg basis.

TCDD was the pilot compound used in several projects which, in the whole, represent a systematic means by which to assess the ability of a chemical to alter host resistance, a vital component of total biological fitness. Effects of TCDD on the developing and adult animal were investigated; a depressed cellular immune response was observed with the degree of depression related to age; immunosuppression did not seem to be a function of hyperactivity of the adrenal gland although early findings in the guinea pig do not substantiate this conclusion; methods which gauge resistance to viral challenge and the physiological functions of polymorphonuclear leukocytes are underway. Chemicals that are immunosuppressive will be incorporated into a project which studies this influence on tumor induction using avian sarcoma virus.

Studies on the comparative toxicopathology of selected hexachlorinated dibenzodioxins are underway. 2,3,7,8-tetrachlorodibenzofuran is also being studied. As additional isomers of these and related chemicals are synthesized and characterized, collaborative studies aimed at defining the induced biological responses as they relate to chemical structure will be pursued. It is hoped that parallel studies which investigate responses on a subcellular level will be realized through collaboration with scientists from other Branches.

Current work with polychlorinated biphenyls is primarily resident within the several chemistry laboratories. Efforts to develop a screening assay for "total" polychlorinated biphenyls in tissue met with limited success. Developing analytical methodology with application to defining PCB exposure through tissue assay will continue; an approach which focuses on specific isomers that are of either primary environmental health consequence or serve as reliable markers for a given PCB formulation is being considered. Hexachlorinated biphenyl isomers were synthesized or purchased, analyzed by a variety of techniques aimed at illuminating biologically significant chemistry, and subsequently evaluated in the baby chick which allowed comparison as to specific toxicity resulting from a dibenzofuran metabolite. Clear indications for toxicity related to isomeric configuration was observed. In general, biological activity (toxicity) correlated with lipophilicity; the pattern of hepatic enzyme response observed with biphenyls were distinct from those produced in birds intubated with a tetrachlorodibenzofuran. Future studies in a mammalian species are planned with the ultimate objective, the identification of isomers of health consequence. We then propose to study those chemical-biological interactive properties that seem to be associated with toxicity.

The Branch has participated extensively in a two-year study with rats exposed to NTA initiated by Dr. Goyer at the University of North Carolina. The various groups have been killed, necropsied, appropriate tissue embedded, cut and stained for histopathological evaluation. This study will contribute in a most material way to evaluating the carcinogenic potential of the compound.

Pasteurella multocida is implicated in a chronic respiratory syndrome of rabbits

that usually manifests itself through a chronic nasal discharge. Extension of the bacterium to lungs, draining lymph nodes or the middle ear often occurs as a result of stress. Morbidity of the disease increases with age with the incidence of the more severe manifestations paralleling the higher infection rate. An attempt to decrease animal loss due to this disease during experiments, by early immunization is underway. An autogenous bacterin was prepared in our Microbiology Laboratory and is being administered to rabbits at time of weaning. Reducing the incidence of P. multocida which often approaches 80% in older rabbits would be a most significant saving of financial and personnel resources.

The applicability of model micellar chemistry towards developing a better understanding of compound interaction at biological sites is being studied. Several chlorinated hydrocarbons were studied. A small but finite hydrophobic catalytic effect for reductive dechlorination of polyclodiene pesticides was demonstrated. Similar effects with mirex, lindane and a chlorinated biphenyl and chlorinated dibenzodioxin isomer were not observed. Further materials are to be studied.

Studies on the transport and metabolism of phthalate esters continued. Only those phthalate esters with some degree of water solubility are absorbed from the intestine; phthalate diesters are hydrolyzed in the intestine to the appropriate alcohol and monoester both of which are absorbed; the monoester is metabolized and excreted in the urine without modification of the phthalate ring: The in vivo metabolism of 2-ethylhexanol is presently being studied.

Work on the metabolism, biosynthesis and interactions of piperidines, pyrrolidines, and allyl benzenes continued. Data indicates that the piperidines and pyrrolidines are not formed by direct decarboxylation of pipecolic acid and proline. Nitrogen metabolites of the allyl benzenes were found to inhibit monoamine oxidase from a variety of tissues.

The NMR spectras of a variety of chemical compounds are being studied. These include aromatic carcinogens, dithiocarbamates, nitrosamines, organomercurials and polychlorinated biphenyls.

Activity toward the development of the opossum colony was primarily that of a holding action in the current year. Earlier work which determined the erythrocyte life span was completed in manuscript form and accepted for publication as was a paper elucidating the means of establishing a helminth-free colony.

Other Activities

Concepts are being discussed with Duke University Medical Center personnel pursuant to establishing a collaborative program in Laboratory Animal Medicine and Comparative Biology. In one phase of the program, NIEHS will co-sponsor the training of qualified individuals at the postdoctorate level. It is felt that collaborative efforts of this type will prove beneficial to all through interchange of thoughts, strengthening of programs and resources that are complimentary and the development of collaborative research relevant to each organization's needs.

Considerable time is spent by various personnel in performing functions that,

while not terribly gratifying, are necessary for the proper function of the intramural program. Nominations to standing committees are accepted without rancor and is usually followed by active participation. Dr. Feldman continues to perform the burdensome role of NIEHS Drug Control Officer. Dr. Albro spends a considerable amount of his time as Chairman of the Institute Editorial Committee.

The Branch Chief currently serves on the HEW Subcommittee on Carcinogen Standards, the HEW Asbestos Protocol Subcommittee and the NIH Biohazards Committee. The HEW Subcommittees have been quite active and command considerable allotments of time.

Serial No.: NIEHS-EBC-001-3
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Health Surveillance of Laboratory Animals at NIEHS"

PREVIOUS SERIAL NUMBER: NIEHS-AST-001-2

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.&A.H., Ph.D., Julius E. Thigpen, Ph.D.

COOPERATING UNITS: Animal Husbandry Section, EBCB

MAN YEARS

Total:	3.5
Professional:	1.2
Other:	2.3

PROJECT DESCRIPTION

OBJECTIVES: To monitor incoming and resident laboratory animals for spontaneous infectious diseases and parasitism.

METHODS EMPLOYED: All research animals are continually observed for clinical signs of disease. Moribund or dead animals on experiment are referred to the investigator to determine if the condition is spontaneous or experimentally induced. Dead, diseased or abnormal animals are usually submitted (following clinical examination) to necropsy where they undergo microbiological and histopathological examination.

New species or strains of animals from new sources undergo health surveillance which includes examination for respiratory and enteric pathogens, parasites, and incidental pathology.

MAJOR FINDINGS: Rats selected at weekly intervals (upon arrival through one month quarantine) have been free of Salmonella, parasites and respiratory pathogens.

Reproductivity of certain inbred strains of mice (C3H, CBA, C57Bl, AKR) has been low. Although Pasteurella pneumotropica has occasionally been cultured from the lungs of these strains, there is no evidence that this organism causes respiratory disease or reproductive decline. This is corroborated by similar findings from the supplier. Inbred strains of mice have a reproduction rate

substantially lower than Swiss mice and are not amenable to intensive reproductive methods. Low reproduction is not due to intercurrent infection.

Guinea pigs are no longer bred at NIEHS, but are purchased as needed from commercial sources. A commercial disease free source has been located.

Approximately 30% of New Zealand White rabbits are infected with subclinical snuffles or Pasteurellosis. Of these, approximately 1/2 will subsequently become overtly ill with pneumonia and/or inner ear infections. The incidence of snuffles in Belted Dutch rabbits is less than 5%.

Chronic diarrhea causes intermittent fatalities among both breeds of rabbits. This syndrome is manifested by either mucoid diarrhea or hemorrhagic colitis. Although E. coli is consistently cultured from the gut of affected animals, its presence is considered secondary to the inciting agent. Recently, Clostridium perfringens has been isolated from the gut. The possibility of anaerobic enterotoxima will be investigated. The mortality rate of affected animals has been reduced approximately 60% by treating rabbits with 50 cc protein hydrolysate-electrolyte solution intraperitoneally at the first sign of infection.

Fatalities among opossums increase sharply from fighting during the mating season with resulting trauma and septicemia. Salmonella and streptococci apparently account for most infections but Pseudomonas, Proteus, Pasteurella and Corynebacterium spp. have also been incriminated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health status of all research animals must be continually monitored; otherwise, intercurrent infections could cause misinterpretation and possible negation of valuable experimental data. In addition, microbiological and histopathological surveillance has uncovered interesting and valuable data heretofore unpublished in laboratory animal medicine.

PROPOSED COURSE: Surveillance of laboratory animals will continue on a routine basis.

PUBLICATIONS

Gupta, B.N. and Feldman, D.B.: Unilateral renal agenesis in the opossum (Didelphis marsupialis virginiana). Lab. Anim. Sci. (In Press)

Gupta, B.N. and Feldman, D.B.: Carcinosarcoma in an opossum. J.A.V.M.A. 163: 586-588, 1973.

Gupta, B.N. and Feldman, D.B.: Epidermoid cysts in opossums. Cornell Vet. 64: 89-93, 1974.

Serial No.: NIEHS-EBC-002
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Organic Synthesis of Environmental Agents and Metabolites (Labeled and Unlabeled) and Development of Organo-Analytical Methodology"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: M. Walker, L. Levy, Ph.D., K. Chae, Ph.D., S. M. dePaul Palaszek, Ph.D. and C. Ford.

COOPERATING UNITS: Institute wide

MAN YEARS

Total:	1.1
Professional:	0.3
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: To provide intra- and interbranch synthetic and radiosynthetic capabilities as well as develop organo-analytical methodology as required to support diverse biological studies with diverse environmental agents and their transformation products.

METHODS EMPLOYED: A variety of synthetic, chromatographic, autoradiographic, and spectroscopic techniques.

MAJOR FINDINGS: The following is a list of the labeled compounds whose syntheses have not been described or implied in other reports of this unit. These syntheses have involved at least one, but generally two, runs with unlabelled starting materials prior to the labeled synthesis:

1. 4,4'-Dichlorobiphenyl-ring labeled, ^{14}C
2. 2,2'-Dichlorobiphenyl-ring labeled, ^{14}C
3. Safrole - allyl side chain labeled, ^{14}C
4. Myristicin - allyl side chain labeled, ^{14}C
5. Elemicin - allyl side chain labeled, ^{14}C
6. Desmethylene aldrin - chlorinated portion, ^{14}C labeled
7. Desmethylene dieldrin - chlorinated portion, ^{14}C labeled
8. Trans-diol of desmethylene dieldrin - chlorinated portion, ^{14}C labeled

9. Cis-diol of desmethylene dieldrin - chlorinated portion, ^{14}C labeled

Other interbranch syntheses have included:

1. 3-N-methyl-5-iodo-2'-deoxyuridine
2. 7-ethoxy coumarin
3. N-oxide of dimethylaniline
4. 4-chloronitrosobenzene - currently being prepared

In complement with these syntheses was development of the necessary analytical methodology to enable isolation, characterization, and identification of the products. Other analytical methods developed which were particularly useful for identification of synthetic products as well as authentic metabolite samples were selected specific ion (mass) monitoring with the gas chromatograph - mass spectrometer (metabolites of hexachlorobiphenyls) and Fourier transform ^{13}C-NMR spectroscopy for determining skeletal changes in polycyclodiene pesticide systems.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Radiolabeled compounds are particularly desirable biological tracers for facilitating diverse biological studies with environmental agents. In addition, new and improved analytical methodology is continually required to enable unequivocal identification with better sensitivity (minimum detectability).

PROPOSED COURSE: To continue to develop new and improved synthetic, radiosynthetic, organo-analytical methodology for preparing and analyzing environmental agents and their transformation products.

PUBLICATIONS

Walker, M., McKinney, J.D. and Oswald, E.: The synthesis of ^{14}C-safrole, myristicin and elemicin labeled in the side chain. J. Labeled Compounds, 1974 (In Press).

McKinney, J.D., Oswald, E.O., Palaszek, S.M. dePaul, and Corbett, B.J.: Characterization of chlorinated hydrocarbon pesticide metabolites of the DDT and polycyclodiene types by electron impact and chemical ionization mass spectrometry. Mass Spectrometry and NMR Spectroscopy in Pesticide Chemistry, Environmental Science Research Series, Vol. 4, F. Biros and R. Haque, Plenum Publishing Corp., New York, N.Y., 1974, pp. 5-32.

Serial No.: NIEHS-EBC-003-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Development of Analytical Methodology"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-006

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D., R. Thomas, J. Fawkes, A. Latimer

COOPERATING UNITS: None

MAN YEARS

Total:	0.50
Professionsl:	0.50
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include (1) those for which there is no analytical methodology, insufficiently specific or sensitive methodology, or methodology we are not equipped to utilize. (2) those which are metabolites of compounds for which adequate analytical methodology is available, and (3) those for which an adequate analytical procedure is available but which occur in a milieu providing interference with the standard procedure.

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UR, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS: (1) Efforts to develop a rapid screening assay for "total polychlorinated biphenyls" in biological tissues based on perchlorination to decachlorobiphenyl achieved only partial success. Mirex can be used as an internal standard, minimizing reproducibility and recovery problems. Unfortunately the requirements for purifying the PCB's prior to perchlorination are so demanding that the number of samples that can be dealt with is too low to consider this an acceptable "rapid screening" method. (2) The identification

of metabolites of phthalate esters by GC-mass spectrometry is greatly facilitated by combining electron impact and methane chemical ionization mass spectrometry, and some of the principles involved appear to have general applicability. (3) Analysis of aminimide samples for Dr. Posner involved a certain amount of time, but did not require developing any new methods.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Section as well as be of utility for other studies within and without the Institute.

PROPOSED COURSE: We will continue to attempt to develop analytical methodology as required under the terms of Objectives above. In particular, an attempt will be made to develop a radioimmunoassay for tetrachlorodibenzodioxins.

Serial No.: NIEHS-EBC-004-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Identification and Characterization of Potentially Hazardous Environmental Agents by Mass Spectrometry"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-033

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: P. W. Albro, Ph.D. and J. D. McKinney, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Our present computerized Mass Spectrometry facilities, including the Finnigan 1015 Electron Impact system and the Finnigan 1015 Chemical Ionization unit afford the capability of collecting and correlating a tremendous amount of spectral information. Because chemical ionization mass spectrometry is most useful in direct complement with electron impact data, all of the compounds are analyzed and correlated on both systems. This catalogued information will serve as an identification library of various organic environmental agents.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), I.R, N.M.R. and mass spectrometry, computer analyses, organic syntheses.

MAJOR FINDINGS: Probably the most versatile analytical technique available is gas-liquid chromatography; however, by this method alone one cannot conclusively verify the chemical structure of a compound. By use of a combined gas-liquid chromatographic system which is directly interfaced to a mass spectrometer, one is able to thoroughly examine the chemical structure of microgram and even low nanogram quantities of environmental agents and their metabolites. In numerous cases because of the polyfunctionality and chemical nature of a compound, even by use of very low energy electron bombardment potentials of 20 volts or less, the mass spectrum may not contain all of the required mass fragments and the molecular ion necessary for positive identification. By use of chemical ionization mass spectrometry combined with gas-liquid chromatography, one is able to obtain the desirable fragment in the area of the molecular ion and other

pertinent mass fragments which unequivocally identify the particular compound even in low nanogram quantities. By the complementary characterization of crudely purified environmental agents from biological samples by combined gas-liquid chromatography with chemical ionization and electron impact mass spectrometry, one is able to identify the chemical structure of many polyfunctional metabolites and reaction products of potentially hazardous environmental agents.

We have applied this complementary use of chemical ionization and electron impact mass spectrometry interfaced with gas liquid chromatography to: (1) chlorinated hydrocarbons including DDT derivatives; polycyclodienes including dieldrin, aldrin and metabolites; and polychlorinated biphenyls (PCB); (2) branched chain hydrocarbons and metabolites including tertiary alcohols; (3) plasticizers including the rat urinary metabolites of di-(2-ethylhexyl)phthalate; (4) prostaglandins; (5) amines and amino acid derivatives; (6) allyl and propenyl benzenes including their urinary metabolites - tertiary aminopropiophenones; and (7) chlorinated dibenzodioxins and dibenzofurans.

Numerous of the above identification problems associated with metabolic investigations would not have been undertaken without this complementary use chemical ionization and electron impact mass spectrometry.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many deficiencies in our knowledge concerning the metabolic fate of phthalates, polychlorinated biphenyls, pesticides, amines, organometallic compounds and numerous other hazardous environmental agents have existed for a number of years. By the use of this powerful analytical tool, chemical ionization and electron impact mass spectrometry, some of these scientific findings have greatly decreased deficiencies of knowledge in these areas.

PROPOSED COURSE: (1) To continue to characterize numerous environmental agents and their metabolites by chemical ionization and electron impact mass spectrometry. (2) To update the mass spectrometer and data retrieval systems. (3) To develop a multiple ion detection capability for quantitative analyses of picogram quantities of specific environmental agents.

PUBLICATIONS

Albro, P.W., Thomas, R., and Fishbein, L.: Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr. 76: 321, 1973.

McKinney, J.D., Oswald, E.O., Palaszek, S.M. dePaul, and Corbett, B.J.: Characterization of chlorinated hydrocarbon pesticide metabolites of the DDT and polycyclodiene types by electron impact and chemical ionization mass spectrometry in mass spectrometry and NMR spectroscopy in pesticide chemistry, Environmental Science Research Series 4, F. Biros and R. Hague (co-chairman) Plenum Publishing Corp., New York, New York, 1974, pp. 5-32.

Serial No.: NIEHS-EBC-005-5
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Toxicity of Dibenzodioxins"

PREVIOUS SERIAL NUMBER: NIEHS-AST-007-4

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D., J. G. Vos, D.V.M., Ph.D., Joseph G. Zinkl, D.V.M., Ph.D., and Martha Harris

COOPERATING UNITS: J. D. McKinney, Ph.D., Chemistry Section, EBCB

MAN YEARS

Total:	1.2
Professional:	0.4
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: Using pure chlorinated dibenzodioxins, determine the dose required to produce a toxic or lethal response in laboratory animals. Identify the sequence of toxicopathological processes associated with selected doses of compound. Investigate if interactions occur in vivo with the dioxin isomers i.e. are there additive, antagonistic or synergistic effects on toxicity.

METHODS EMPLOYED: Rats, mice and guinea pigs were given single or multiple doses of TCDD orally in order to study the toxicologic, pathologic and clinical pathologic effects of the intoxication. Standard techniques were used in order to evaluate these effects.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HCDD) are currently being evaluated.

MAJOR FINDINGS: The toxicopathology of TCDD in C57Bl/6 mice was studied. The single oral LD_{50} was found to be 117 µg/Kg with thymic and splenic atrophy consistently observed. Degenerative and necrotic liver changes were observed at the higher doses. Proliferation of bile duct and bile duct epithelial cells occurred with time.

The toxicopathology of HCDD in mice is currently being evaluated. Although most of the tissue and data await evaluation, it is apparent that the LD_{50} for this hexachloro isomer is between 500 + 1000 µg/Kg and that thymus and liver are

the organs showing a pathological effect.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins may occur in association with many useful chlorinated cyclic compounds. The symmetrical tetrachloro isomer is one of the most toxic chemicals known. The pattern of toxicity associated with most of the isomers has not been determined. The interactions of isomers as they relate to toxicity is unknown. These facts must be determined if assessment of the environmental health implication of products which contain dibenzodioxin isomers as contaminants are to be made.

PROPOSED COURSE: To be continued.

PUBLICATIONS

Harris, M.W., Moore, J.A., Vos, J.G. and Gupta, B.N.: General biological effects of TCDD in laboratory animals. Environmental Health Perspectives, Experimental Issue No. 5, September 1973, pp. 101-109.

Gupta, B.N., Vos, J.G., Moore, J.A., Zinkl, J. G., and Bullock, B.C.: Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environmental Health Perspectives, Experimental Issue No. 5, September 1973, pp. 125-140.

Vos, J.G., Moore, J.A. and Zinkl, J.G.: Toxicology of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57Bl/6 mice. Toxicology and Applied Pharmacology. (In Press)

Serial No.: NIEHS-EBC-006-3
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin"

PREVIOUS SERIAL NUMBER: NIEHS-AST-023-2

PRINCIPAL INVESTIGATOR: John A. Moore, D.V.M.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D., J. G. Vos, D.V.M., Ph.D. and Martha Harris

COOPERATING UNITS: Dr. Phillip Albro, Chemistry Section, EBCB

MAN YEARS

Total:	1.2
Professional:	0.4
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: Characterize the developmental stages that are susceptible to TCDD. Attempt to determine the mechanisms by which these effects are produced.

METHODS EMPLOYED: Using rat and mouse experimental models, characterize the type and degree of organ pathology produced and, in selected instances, determine functional efficiency of appropriate organ systems. Administer C^{14} labeled TCDD to the maternal animal and determine absorption, distribution, placental transfer, excretion, secretion and metabolism in the adult, fetus and neonate. Determine nucleic acid content of mammary glands from nursing rats to assess function.

MAJOR FINDINGS:

(1) TCDD is found to effect placental transfer albeit at comparatively low levels. Marked quantities of TCDD are secreted in the milk. No metabolites of TCDD have been observed. The majority of this work is still in progress.

(2) Pregnant rats were given TCDD orally on days 11 and 18 of gestation, at levels of 3.0, 1.0 or 0.0 µg/Kg body weight. Nucleic acid content of mammary glands were determined 1 day before, and 3 and 10 days after whelping. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of mammary gland were significantly decreased 3 days after whelping. RNA/DNA ratios were also decreased in rats 10 days after whelping at 3 µg/Kg level. Decreased RNA in the mammary

gland is suggestive of depressed functional activity (less milk secretion), thereby causing caloric deprivation to the nursing pups.

(3) Studies on the significance of reduced weight and cortical atrophy of thymus is a separate report. (See Effects of Environmental Chemicals on Immunocompetence, NIEHS-EBC-014-2).

(4) Maternal exposure to TCDD in mice produces hydronephrosis in offspring as reported in previous years. Further characterization of this anomaly is currently suspended.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: TCDD is one of the most toxic chemicals known. Its ability to produce anatomical and functional defects in young at doses which do not cause maternal effects have been demonstrated. Apparent favored secretion in milk has obvious implication as to effects on suckling young and those who may consume milk obtained from TCDD exposed animals.

PROPOSED COURSE: Project to continue.

PUBLICATIONS

Moore, J.A., Gupta, B.N., Zinkl, J.G., and Vos, J.G.: Postnatal effects of maternal exposure to TCDD. Environmental Health Perspectives, Experimental Issue No. 5, September 1973, pp. 81-85.

Serial No.: NIEHS-EBC-007
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolism of Dioxins and Dibenzofurans"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Edward O. Oswald, Ph.D.

OTHER INVESTIGATORS: Phillip W. Albro, Ph.D., Jean Corbett

COOPERATING UNITS: John A. Moore, D.V.M., Martha Harris, Comparative Biology Section, EBCB

MAN YEARS

Total:	0.4
Professional:	0.1
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To study the absorption, tissue distribution, subcellular distribution and metabolism of dibenzodioxins and dibenzofurans in laboratory animals, in order to facilitate evaluation of their modes of action.

METHODS EMPLOYED: [^{14}C]-2,3,7,8-tetrachlorodibenzodioxin of high specific activity is administered to laboratory animals for subsequent study of tissue distribution, uptake and excretion, and subcellular localization by common techniques. The enrichment technique is used in attempts to isolate soil, air and fecal microbes capable of metabolizing dibenzodioxins and dibenzofurans. Metabolism of ^{14}C-TCDD is detected by radio-GLC and radio-TLC monitoring of tissue or fecal extracts.

MAJOR FINDINGS: (1) We have been unsuccessful in attempts to isolate any soil or fecal bacterium capable of utilizing dibenzofuran as a carbon source for growth. (2) High-specific-activity ^{14}C-TCDD has chromatographic (and mass-spectral) properties different from those of unlabeled TCDD. (3) The liver and feces from rats given ^{14}C-TCDD orally 72 hours previously were examined for labeled metabolites. None were detected in liver (only unchanged ^{14}C-TCDD) but at least 80% of the ^{14}C in feces collected $\sim$60 hours after feeding TCDD was in the form of metabolites. Radioactivity in liver was recovered from all major subcellular fractions, with microsomes being highest on a dpm/mg protein basis. However, nuclei were highest on a dpm/mg lipid basis, and mitochondria were highest on a dpm/mg fat basis. (4) Aerobic rat fecal bacteria did not metabo-

lize ^{14}C-TCDD in artificial media.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The extremely high affinity of rat liver for TCDD, without evidence for liver metabolism of TCDD, and the presence of TCDD metabolites in rat feces but not in cultures of fecal bacteria are aspects of TCDD biological interactions that may have significance relative to species differences in sensitivity and types of pathology, but further studies will be needed to evaluate this.

PROPOSED COURSE: Distribution/metabolism studies should be performed in species other than rat. If a species is found that can tolerate significant doses of TCDD, an attempt can be made to identify metabolites. These studies should be extended to include (a) non-toxic isomers of TCDD and (b) tetrachlorodibenzofurans if appropriate ^{14}C-labeled compounds become available.

Serial No.: NIEHS-EBC-008
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Comparative Chemistry and Biological Activity of Selected Oxygenated Chloro-Aromatics, Dibenzodioxins and Dibenzofurans"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: K. Chae, Ph.D., L. Levy, Ph.D., and P. Albro, Ph.D.

COOPERATING UNITS: J. A. Moore, D.V.M. and B. N. Gupta, Comparative Biology Section

MAN YEARS

Total:	0.8
Professional:	0.8
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To elaborate the chemistry of representative compounds, such as the hydroxy chlorobiphenyls, chlorinated dibenzodioxins and furans, the predioxins and prefurans, etc., which are of environmental health concern as possible degradation products or contaminants of man-made chlorinated hydrocarbons. To utilize this chemistry in connection with toxicological studies in gaining an understanding of their biological properties, mechanism and mode of action, etc.

METHODS EMPLOYED: A variety of synthetic, chromatographic and spectroscopic techniques aimed at illuminating biologically significant chemistry. Relative toxicological studies (LD_{50}'s, etc.) of the individual compounds in various mammaliam systems.

MAJOR FINDINGS: Synthetic methods have been developed for preparation of various substituted hydroxy hexachlorobiphenyls, some of which are prefurans, which are the suspected metabolites isolated from recent toxicological studies with the parent hexachlorobiphenyls in the chicken.

Efficient synthetic methods have also been developed for the preparation of the highly toxic 2,3,7,8-tetrachloro- and 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin. The 2,8-dichlorodioxin has also been prepared from a commercial product, Irgasan DP300. Penta- and heptachlorodioxins occur as by products in these syntheses

but have not been isolated and fully characterized. The corresponding penta- and heptachloropredioxins are also by products and exhibit sufficient stability of their own to enable one to encounter them as contaminants of commercial products. Irgasan DP300, a predioxin failed to undergo in vitro conversion to the dioxin (in rat liver tissue).

Other synthetic methods have centered on the preparation of 1-amino-2,3,7,8-tetrachlorodibenzodioxin for subsequent conversion to the diazonium salt or an equivalently activated species for covalent linking to serum albumin. This would hopefully enable the development of a sensitive radioimmunoassay technique for the highly toxic 2,3,7,8-TCDD. In the course of these studies, attempts to directly nitrate TCDD (H_2SO_4-HNO_3) produced colored solutions reminiscent of the colored solutions attributed to radical cation formation under acidic oxidizing conditions. The TCDD is readily destroyed under these conditions, but the desired nitro TCDD derivative was not found among the products. The cation radical intermediate is apparently subsequently further degraded with a corresponding color change. TCDD may not be susceptible to electrophilic attack at the 1,4 or 6,9 positions. A directed synthesis is being sought in which the appropriate amino trichlorophenol will be reacted under basic conditions with the normal trichlorophenol used in TCDD synthesis. The desired amino trichlorophenol appears to be in hand.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Members of the chlorinated dibenzo-p-dioxins and furans are among the most toxic small molecules known to man. Various ones and their precursors (predioxins and prefurans) have been identified as contaminants in commercial products as well as implicated in the environmental degradation of certain chlorinated hydrocarbons. Therefore, it is necessary to obtain an understanding of the bioorganic chemistry of these systems in order to ascertain whether or not exposure to or ingestion of these agents constitutes a potential environmental health hazard.

PROPOSED COURSE: To further elaborate the bioorganic chemistry of these oxygenated chloroaromatics and systematically determine the toxicological properties of these compounds in light of the chemical information obtained.

Serial No.: NIEHS-EBC-009-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Synthesis and Chemistry of Chlorinated Aromatic and Oxygenated Chlorinated Aromatic Compounds and Their Environmental Transformation Products"

PREVIOUS SERIAL NUMBER: NIEHS-AST-035

PRINCIPAL INVESTIGATOR: Louis A. Levy, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D., M. Walker, J. D. McKinney, Ph.D.

MAN YEARS

Total:	1.1
Professional:	1.0
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To develop rational synthetic routes to chlorinated biphenyls, chlorinated dibenzofurans and their metabolites, especially the assumed reactive intermediate arene oxides (oxepins) and penultimate hydroxylated derivatives. To investigate the chemical and physical properties of these compounds and their interaction-reaction products, which will aid in identifying them in the environment and assessment (rate and extent) of reactivity with biological entities.

METHODS EMPLOYED: Synthetic techniques; organometallic reagents and catalysis, high pressure reactions, photochemicals processes, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS: A number of polychlorinated biphenyls have been prepared in a homogeneous state. Included in this group are those which have been found to be present in commercial Aroclors and/or also found to be present in the environment. Also prepared were polychlorinated biphenyl isomers not expected on theorecteral grounds, to be present in the commercial mixture but found to possess potent biological activity.

A convenient preparation of pure 2,3,6 trichlorobenzoic acid has been developed incidental to the synthesis of some of these isomers. This material, approximately judging from the literature, is widely used in herbicide formulations, but has been available only with difficulty in a homogeneous state.

Significant progress has been obtained in the correlation of mass spectral fragmentation with isomer structure. Depending on the degree of ortho substitution more or less characteristic spectra of various isomers have been obtained along with some capability of predicting structure.

The use of organo lithium reagents to reductively remove chlorine atoms, one at a time, from polychlorinated biphenyls has had some success. This procedure should allow for preparation of compounds containing an odd number of chlorine atoms from the more readily available chlorinated compounds with an even number of chlorine atoms.

The synthesis of some hydroxylated hexachlorobiphenyls has been effected. These compounds represent very likely metabolites of the precursor hexachlorobiphenyl. The aforementron mass spectral correlations were of some help in assigning structures to the mixture of products obtained from this preparation.

The use of the Diels-Alder cycloaddition reaction has been explored as an aid in the synthesis of biphenyls and dibenzofurans (a biphenyl derivative). While this work is still in the early stages, it does seem to represent an extremely promising general synthetic method. An advantage of this approach is that the biphenyl nucleus is formed in the first step of the synthetic scheme along with the proper functionality to eventually afford the desired compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The commercial PCB's are known to be complex mixtures and are recognized to be of major environmental importance due to their widespread ecological distribution and their concentration in the food chain. Furthermore, chlorinated dibenzofuran impurities found along with PCB and/or produced from PCB by natural processes are known to be extremely toxic. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit more definitive biological and toxicological studies.

PROPOSED COURSE: To continue the synthesis of individual isomers of PCB's and dibenzofurans as well as their potential or actual metabolites such as arene oxides and hydroxylated derivatives. Especial attemtion is to be focused on the continuing use of the cycloaddition reaction and associated sequence mentioned above.

PUBLICATIONS

Levy, L.: Synthesis of Some Chlorinated Biphenyls. Synthesis 170 (1973).

Serial No.: NIEHS-EBC-010
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Chemistry and Biological Properties of Selected Isomeric Hexachlorobiphenyls"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: James D. McKinney, Ph.D.

OTHER INVESTIGATORS: K. Chae, Ph.D., J. A. Moore, D.V.M., L. Levy, Ph.D., M. Walker and J. Goldstein, Ph.D.

COOPERATING UNITS: Pesticides and Toxic Substances Effects Lab, NERC, EPA, Research Triangle Park, N.C. 27711

MAN YEARS

Total:	1.2
Professional:	1.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To begin to clarify and define the biological effects of commercial PCB-mixtures by systematically examining the chemistry and biological effects of individual selected isomeric hexachlorobiphenyls.

METHODS EMPLOYED: Defining the in vivo and in vitro chemistry of purified synthetic hexachlorobiphenyls via a variety of synthetic, chromatographic and spectroscopic techniques. Simultaneous measurement of such biological responses as mortality, body weight change, food consumption, body organ weights, incidence of edema, enzyme induction, porphyria, etc.

MAJOR FINDINGS: The biological potency of five purified hexachlorobiphenyls [symmetrical isomers, 245, 236, 246, 234 and 345 (contained about 30% hepta)] and 2,3,7,8-tetrachlorodibenzofuran (TCDF) was investigated in the chicken at a dietary level of 400 ppm (except 245 @ 100 or 400 ppm; TCDF @ 1 or 5 μg/Kg) over a 21 day period. The chicken was chosen for the initial studies since it was thought in some ways to be a more sensitive indicator of relative toxicities. There was generally an increase on biological potency with increasing lipophilicity as measured by retention indexes on gas chromatography with the notable exception of the 246 isomer which was more potent than predicted on this basis. Nevertheless, the 345 isomer was generally the most effective (all chicken died) and the 236, the

least. Measurement of the induction (lack of an inhibitor) of liver enzymes, P-450 levels, etc. enabled a clear differentiation between a hexachlorobiphenyl and TCDF effect. It is also known that a small, but finite, amount of metabolism of the hexachlorobiphenyls occurs to produce in some cases both mono and dihydroxylated products that can lead to p-quinones, hydroxy-dibenzofurans or both.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use and occurrence of the polychlorinated biphenyls has necessitated investigations of their possible human health effects. In order to facilitate such studies, systematic examination of purified isomers is necessary to obtain clarity and definition of the biological effects.

PROPOSED COURSE: To extend studies with the chicken to include the more toxic (biologically potent) hexa-isomers and their possible oxygenated metabolites which may be of greater biological consequence. To extend studies to the mammalian system.

Serial No.: NIEHS-EBC-011-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolism of Polychlorinated Biphenyls"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-036

PRINCIPAL INVESTIGATORS: Phillip W. Albro, Ph.D.

OTHER INVESTIGATORS: E. O. Oswald, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.0
Professional:	0.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the mammalian and microbial metabolism of labeled and unlabeled chlorobiphenyls.

METHODS EMPLOYED: Chromatographic, mass spectrometric, and radio-tracer techniques.

MAJOR FINDINGS: As appropriate ^{14}C-labeled PCB's have not become available, we have not progressed with this project this year.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: No results = no significance.

PROPOSED COURSE: Currently suspended.

Serial No.: NIEHS-EBC-012-3
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Effect of Acute Starvation after Long-Term, Low-Level Feeding of a Polychlorinated Biphenyl Mixture (Aroclor 1254) in Rats.

PREVIOUS SERIAL NUMBER: NIEHS-AST-020-2

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.15
Professional:	0.10
Other:	0.05

PROJECT DESCRIPTION

OBJECTIVES: To study the effects of starvation induced mobilization of PCB's stored in body fat.

METHODS EMPLOYED: Female rats weighing approximately 175 gms were fed Aroclor 1254 in the diet at 10, 30, and 100 ppm. After 2, 5, 8, 12 and 16 weeks five animals in each group were bled and killed for clinical pathological and histopathological evaluation. After 20 weeks, one-half of the remaining animals were starved and one-half were given rat laboratory chow free of PCBs. Three, 6, 10 and 13 days later 4 or 5 rats from each group were bled and killed for evaluation.

MAJOR FINDINGS: Histopathologic lesions seen during the first 20 weeks of the study were confined to the skin and liver. Approximately 15 animals in the high-dose groups (100 ppm) developed scab-like lesions on the ears. Some also had this lesion on the nose, feet and tail. These lesions consisted of keratin plugging of follicles, hyperkeratosis and parakeratosis. Liver lesions at 16 weeks consisted of cellular degeneration, mild focal inflammation, megalocytosis, and accumulation of brown pigment in Kupffer cells. Porphyria was noted by florescence under ultraviolet light.

After starvation liver lesions were seen in all the animals which had previously received PCBs. These consisted of focal necrosis, cellular degeneration,

inflammation, bile duct proliferation (also seen in non-starved animals), megalohepatocytosis and multinucleated cells. However, the focal necrosis cellular degeneration and the acute inflammatory response were confined to starved animals which had previously received PCBs.

Clinical pathologic changes were increased serum cholesterol, SGOT and β-globulin and decreased γ-globulin in the first 20 weeks of the study. After starvation, SGPT and SGOT activities were markedly increased in the starved animals which received PCBs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The skin lesions seen in these animals suggest the chloracne can be caused by PCBs even at a relatively low dose. Perhaps this lesion has not been seen previously because there may have been one or more isomers in this lot of Aroclor 1254 which had an extremely detrimental effect on skin and which may not have been found in other lots used by other investigators.

The production of liver lesions after starvation in animals previously fed low levels of PCBs for a long period of time suggests that the PCBs were mobilized from fat tissue to cause the lesions. This effect suggest that a continuous low dose of PCBs (and perhaps other lipid soluable chlorinated hydrocarbons) could be very toxic if the animal (man or others) are subsequently subjected to acute starvation such as during famines, anorexia of illness, crash diets, hibernation, and natural periodic depletions of food supplies.

PROPOSED COURSE: Manuscripts describing the skin lesions and the acute starvation induces liver lesions are being written. Tissue analyses for PCBs are planned in order to document the hypothesis that the PCBs move from fat stores to the liver during acute starvation.

Serial No.: NIEHS-EBC-013-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Nuclear Magnetic Resonance Studies of Substituted Biphenyls"

PREVIOUS SERIAL NUMBER: NIEHS-AST-037

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: Marshall Anderson, Ph.D.

COOPERATING UNITS: Biometry Branch

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To study the detailed molecular and electronic structure of several substituted biphenyls and their possible interactions with complexing agents which may be of biological importance.

METHODS EMPLOYED: Variable temperature NMR and supportive computer methodology.

MAJOR FINDINGS: The relationship between NMR parameters and calculated electron densities in several symmetric and asymmetric chlorinated biphenyls have been found. Preferred conformations have been predicted for some of the biphenyls. Spin-lattice relaxation time data have been used to investigate the molecular conformations and motions of some of these compounds. Complex formation between the chlorinated biphenyls and some typical complexing agents has been found to be unimportant.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies should help to illuminate the types of interactions in which the substituted biphenyls can participate. The polychlorinated biphenyls, in particular, are of wide current interest as environmental hazards. This work should increase our understanding of how they may be biologically active.

PROPOSED COURSE: Completion of this project within the next few months is anticipated.

PUBLICATIONS

Wilson, N.K. and Anderson, M.: Carbon-13 and proton magnetic resonance studies of chlorinated biphenyls, in Mass Spectrometry and NMR in Pesticide Chemistry, Plenum, New York, 1974, pp. 197-218.

Serial No.: NIEHS-EBC-014-2
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effect of Environmental Chemicals on Immunocompetence"

PREVIOUS SERIAL NUMBER: NIEHS-AST-031

PRINCIPAL INVESTIGATOR: J. G. Vos, D.V.M., Ph.D.

OTHER INVESTIGATOR: John A. Moore, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	1.6
Professional:	0.6
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: To develop and assess various methods for determining humoral or cellular immune capabilities as modulated by exposure to environmental agents.

METHODS EMPLOYED: Cell-mediated immunity is assessed in vivo by the following procedures: delayed type hypersensitivity, graft versus host activity, allograft rejection and in vitro by mitotic response of lymphocyte or thymus cultures to PHA or Con A. Humoral immunity is determined by radial immunodiffusion or passive hemagglutination methods. Other parameters (adrenal function, caloric deprivation, general toxicity) are also observed to determine their influence on the immune response.

MAJOR FINDINGS: Current research addressed itself to the effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the fetus' and neonates' developing cellular immune system. TCDD does depress the cellular immune capabilities in rats and mice. In vitro response of thymus cells to Con A and PHA was different (depressed response to PHA on a cell-for-cell basis with little depression observed in response to Con A) suggesting that specific subsets of cells may be more susceptible to TCDD than others. Mouse experiments indicate that the degree of immunosuppression observed is related to age.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is important to establish possible effects of environmental contaminants on the immune system, and to determine the mode of action, in order to evaluate the hazards of immune suppression for man and animal.

PROPOSED COURSE: To be continued.

PUBLICATIONS

Vos, J.G., Moore, J.A., and Zinkl, J.G.: Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environmental Health Perspectives, Experimental Issue No. 5, September 1973, pp. 149-162.

Serial No.: NIEHS-EBC-015
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Functional Capabilities of the Polymorphonuclear Leukocyte from Animals Treated with Environmental Chemicals"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M, Ph.D.

OTHER INVESTIGATORS: Julius Thigpen, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: A variety of environmental compounds have been shown to decrease the resistance of organisms to infectious diseases. Here-to-fore most efforts to detect the mechanism of this phenomonen have been directed toward the immunosuppressive effects of the compounds. However, there are many other defense mechanisms used by an organism to resist disease. Among these are the functions of the polymorphonuclear leukocyte; namely its chemotactic, phagocytic and bacteriocidal abilities. This study is designed to study these functions in cells from animals exposed to environmental chemicals.

METHODS EMPLOYED: Initial efforts will involve polymorphonuclear leukocytes obtained from guinea pigs treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Leukocytes will be isolated from suitably treated animals. Chemotaxtic ability will be tested in vitro by causing polymorphenuclear leukocytes to migrate through millipore filters toward a solution of E. coli endotoxin. Phagocytic ability will be tested by putting polymorphonuclear leukocytes and bacteria (usually a staylococcus) together and incubating. After a period of time the cells will be isolated, smeared, stained and the number of bacteria ingested determined. Bacteriocidal ability will be determined similarily except that the cells will be lysed to free the bacteria and plate counts performed to determine the number of viable bacteria remaining.

MAJOR FINDINGS: This project is just beginning.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Resistance to disease causing organisms is a multifactorial phenomenon. Although immunologic mechanism are vitally important, other mechanisms are equally important. Therefore, it is necessary to determine what affect environmental agents might have on the functions of polymorphonuclear leukocytes. This will begin to put more of the mechanisms concerned with disease resistance in perspective when the subject of environmental induced alterations to disease resistance is considered.

PROPOSED COURSE: Study is beginning.

Serial No.: NIEHS-EBC-016
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effects of Immunosuppressive Environmental Compounds on Intracerebral Tumor Induction by Avian Sarcoma Virus"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Dana D. Copeland, M.D.

OTHER INVESTIGATORS: none

COOPERATING UNITS: Darell D. Bigner, M.D., Ph.D., Department of Pathology, Duke University

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To define a suitable model for tumor induction in laboratory animals by a transmissible biological agent and to assess any effect of selected environmental compounds on the oncogenic activity of the agent.

METHODS EMPLOYED: The B-77 strain of avian sarcoma virus is grown in tissue culture of ckick embryo fibroblasts. Virus is isolated and then concentrated by filtration and centrifugation. Inocula of virus in citrate buffer are delivered by microliter syringe intracerebrally into fetal, neonatal, and adult Fischer 344 rats. Immunosuppressive agents including anti-thymocyte serum are administered before and/or after administration of virus. Following treatment animals are followed for up to 150 days post inoculation. Animals that die or survive are autopsied and the presence of tumors determined by microscopy.

MAJOR FINDINGS: B-77-ASV induces cerebral tumors in adult as well as perinatal rats. The percentage of tumor induction and the mean number of tumors per animal decreases as the age of the animal at inoculation increases. Tumor related mortality is most precipitous in rats inoculated at younger ages and is more precipitous in male than in female rats inoculated as adults. After virus administration infectious virus can not be recovered from the brain of the treated animal and no tumor is evident. After a latent period tumors appear, but infectious, extracellular virus is not present. The length of the latent period increases with increasing age of the animal at inoculation and decreasing

viral titer. At very low titers the latent period may exceed the normal life-span of the animal.

A large majority of tumors induced in all age groups are glial: the remainder are mesenchymal and mixed glial and mesenchymal. Inoculation in utero and at one day of age induces mixed gliomas with populations of oligodendroglia, astrocytes and spongioblasts. Tumors in groups inoculated as adults include: gemistocytic, pilocytic, fibrillary and anaplastic astrocytomas and only rare mixed tumors. No neuronal tumor has been encountered even in animals inoculated in utero at a time when neurons are still dividing.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental agents which may not in themselves be oncogenic may potentiate oncogenic viruses. An evaluation of suppression of immune surveillance mechanisms which may regulate the oncogenic activity of a virus is an important part of the assessment of any potentially hazardous agent. The eperimental model described above provides an efficacious technique for the ellucidation of such effects.

PROPOSED COURSE: Work is now in progress to define the effects of cellular immunosuppression in perinatal and adult animals. Once baseline data is established trial experiments combining treatment with virus and known immunosuppressive environmental agents will be undertaken. Plans are also being formulated to explore the endocrinological basis of the difference in response of female and adult rats.

Serial No.: NIEHS-EBC-017
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Effects of Environmental Chemicals on Host Resistance to Infectious Agents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Julius E. Thigpen, Ph.D.

OTHER INVESTIGATORS: John A. Moore, D.V.M.

COOPERATING UNITS:

MAN YEARS

Total:	0.4
Professional:	0.1
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: 1. To develop test systems which determine the effects of environmental pollutants on disease resistance of laboratory animals as measured by mortality rates subsequent to exposure to infectious agents.

METHODS EMPLOYED: Pseudorabies virus (PRV) will be produced in quantity using rabbit kidney (RK-13) monolayers and stored in aliquots at -70°C until ready for use. A lethal dose response curve will be established in different age groups of C_3H/HEN mice.

Mice treated with environmental chemicals will be challenged with PRV. An equal number of normal mice (controls) will be infected with the same dilution of virus. Morbidity and mortality rates will be compared.

Another procedure will involve vaccination of treated and control mice prior to challenge with PRV. Resistance to challenge will be a means of determining immune competence.

MAJOR FINDINGS: Study just starting and are now determining dose curves in healthy mice. C_3H/HEN mice (5-6 month-old) are susceptible to PRV infection, but require more than 1000 units of infectious virus (as determined by Plaque assay) for a lethal dose. Mice die within 3 to 10 days depending on concentration of PRV. The LD_{50} dose appears to be about a 1:4000 dilution of the stock virus. Plaque and TCID assays of the stock virus are 1.0×10^7 units per ml.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental chemicals have been shown to affect the immune system of laboratory animals. Both cellular and humoral immunity have been suppressed. Exposure to pollutants or toxicants may render laboratory animals and possibly humans more susceptible to infective agents. These studies will attempt to define the degree of interrelationship that exists between exposure to environmental chemicals and increased susceptibility to virus exposure.

PROPOSED COURSE: To continue.

Serial No.: NIEHS-EBC-018
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Adrenal Gland Function of Guinea Pigs after Receiving 2,3,7,8-Tetrachlorodibenzo-p-dioxin"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl, D.V.M., Ph.D.

OTHER INVESTIGATORS: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D., John A. Moore, D.V.M., J. G. Vos, D.V.M., Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.35
Perfessional:	0.20
Other:	0.15

PROJECT DESCRIPTION

OBJECTIVES: Thinning of the zona glomerulosa of guinea pigs has been seen following administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Since this area of the adrenal gland produces aldosterone it was thought that the production of this hormone might be depressed such that an electrolyte imbalance could occur and cause these animals to die.

A second observation was that humoral and cell-mediated immunity are depressed in guinea pigs given TCDD. These alterations are thought not to be secondary to a hyperadrenal cortical activity, but only exacting measurements of the serum cortisol levels can rule out this effect.

METHODS EMPLOYED: Guinea pigs weighing about 200 gm were divided into 2 groups. Animals of the first group received an oral dose of 3 µg TCDD/Kg B.W. (previously shown to be a lethal dose). The second group of animals received either 0.2 or 0.04 µg TCDD/Kg weekly for 8 weeks. Controls were given only the vehicle in which the TCDD was suspended.

Twenty four hour urine samples were collected from all animals once a week for determination of Na^+, K^+ and aldosterone excretion. The 3 µg TCDD/Kg guinea pigs were bled for cortisol determination at 10 and 14 days after receiving TCDD. Tissues were collected for histopathologic evaluation at this time.

MAJOR FINDINGS: The guinea pigs given 3 μg/Kg had serum cortisol values which were 8 times the values of their controls at both 10 days and 14 days after receiving the initial dose. Their urinary 24 hour aldosterone excretions were similar to those of the controls. Urinary Na^+ and K^+ excretions were 1/3 those of the controls. The rest of the data is in the process of being completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These results suggest that some of the immunosuppressive effects of TCDD in guinea pigs might be due to increased cortisol production secondary to the stress of the toxic compound. The apparent atrophy of the zona glomerulosa in guinea pigs given TCDD appears not to be functionally significant. The lowered Na^+ and K^+ excretions in the guinea pigs given TCDD is likely a reflection of decreased food consumption.

This project is exploring one possible mechanism which could cause death in animals exposed to TCDD.

PROPOSED COURSE: The project will continue in an effort to analyze serum aldosterone levels and determine the role that altered adrenal function might play in the death of guinea pigs given TCDD.

Serial No.: NIEHS-EBC-019
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Carcinogenicity of Trisodium Nitrilotriacetate (NTA) in the Rat"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Robert A. Goyer, M.D., University of North Carolina, Chapel Hill

OTHER INVESTIGATORS: Donald B. Feldman, D.V.M.

COOPERATING UNITS: Comparative Biology Section, EBCB and Clinical Laboratories, Memorial Hospital, University of North Carolina, Chapel Hill, N.C.

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the incidence of tumors in rats fed low levels of NTA in the drinking water throughout most of their life.

METHODS EMPLOYED: Male Sprague Dawley rats (50-70 g) were divided into 2 groups of approximately 200 animals each and were housed individually in suspended cages in a barrier sustained room. The control group recieved deionized drinking water containing 0.1% NTA (100 ppm). Those rats that died, were moribund, or continually lost weight during the experimental period were necropsied. Histopathologic examination was performed on major organs to determine the cause of death or morbidity. Rats with discernible tumors were also necropsied. Approximately 26 months after commencement of the experiment, all survivors were killed and necropsied as above. Blood samples were taken from approximately 180 control or experimental rats for selected clinical chemistry determination. Femurs and sternae from 40 rats were frozen, to be subsequently analyzed for NTA content.

MAJOR FINDINGS: Examination of tissue and neoplasms is currently being performed. Blood sera have been submitted for analyses.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTA

usage in commercial detergents has been delayed in this country until definitive studies determine its safety, including assessment as to carcinogenic potential. The results of this study hopefully will contribute to the final decision.

PROPOSED COURSE: Publication of data.

Serial No.: NIEHS-EBC-020
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Evaluation of a Combined Pasteurella-Bordetella Autogenous Bacterin in Rabbits"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Julius E. Thigpen, Ph.D.

OTHER INVESTIGATORS: Donald B. Feldman, D.V.M., John A. Moore, D.V.M.

COOPERATING UNITS: Pel-Freez Rabbitry, Animal Husbandry Section, EBCB

MAN YEARS

Total:	0.50
Professional:	0.20
Other:	0.30

PROJECT DESCRIPTION

OBJECTIVES: To produce and evaluate a combined Pasteurella multocida - Bordetella bronchiseptica autogenous bacterin against respiratory disease ("snuffles") in rabbits.

METHODS EMPLOYED: Pasteurella multocida (Serotypes A, B, D, E) and Bordetella bronchiseptica isolates from infected New Zealand White rabbits were grown separately in trypticase soy broth. Total counts were determined on each serotype after which they were formalized and tested for sterility and toxicity. Approximately 200 cc of bacterin and control (placebo) respectively were submitted to the rabbitry to be administered in a blind study to female weanling rabbits 8 weeks old. The initial 2 cc vaccination was followed by 1 cc 2 weeks later. These animals were approproately tattooed A or B depending upon the vial label (A=vaccine; B=placebo) and allowed to mature until 6 month of age. Upon return to NIEHS, they will be cultured and observed closely for clinical signs of respiratory disease prior to and during the experimental period. The effectiveness of the vaccine may then be evaluated.

MAJOR FINDINGS: The bacterin is currently being administered. The first vaccinated rabbits are due in approximately 3 months.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Approximately 70-80% of incoming NZW rabbits harbor Pasteurella multocida and Bordetella bronchiseptica in their nasal passages. Those animals succumbing

to respiratory disease (pneumonia, pleuritis, otitis internal) have almost invariably been positive for P. multocida. Antibiotics have been found to be ineffective against rabbit "snuffles"; moreover, antibiotics cannot be used because of the nature of research at NIEHS. Vaccination is acceptable and if effective, can significantly reduce the loss of valuable experimental animals.

PROPOSED COURSE: Evaluation of the bacterin and publication of the results.

Serial No.: NIEHS-EBC-021
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Application of Micellar Chemistry to the Study of (1) Micro-environment of Enzyme Active Sites and (2) Biological Membranes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: J. D. McKinney, Ph.D.

OTHER INVESTIGATORS: S. M. dePaul Palaszek, Ph.D., and Cecil Ford

COOPERATING UNITS:

MAN YEARS

Total:	1.3
Professional:	1.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To investigate the utility of the micellar association of diverse environmental agents of the chlorinated hydrocarbon type as a relatively simple model system for hydrophobic interactions in globular protein enzymes and for lipid-lipid and other lipid protein interactions on complex systems such as biological membranes.

METHODS EMPLOYED: A variety of synthetic, chromatographic, autoradiographic, spectroscopic techniques including proton magnetic resonance and mass spectrometry aimed at determining the effects of micells on reaction kinetics and complex equilibria.

MAJOR FINDINGS: A small but finite hydrophobic catalytic effect has been demonstrated for the reductive dechlorination of several chlorinated hydrocarbon pesticides of the polycyclodiene type (dieldrin, endrin, etc.) utilizing dodecylammonium acetate (or benzoate) as a cationic surfactant in refluxing p-dioxane. Similar studies using mirex, lindane, decachlorobiphenyl, and 1,2,3,4-tetrachlorodibenzodioxin gave unchanged substrates suggesting that the dechlorination reaction may require both hydrophobic and hydrophilic functions in the molecule spaced 6-7A° apart. In this regard, the dechlorination reaction is regiospecific and stereoselective, both characteristics found in enzyme reactions. The existence of a micelle structure in dioxane cationic surfactant could not be measured definitely by spectroscopic or potentiometric methods. Therefore, it has not been determined if this is truly a micellar effect on

the entropy of the reaction or a simple hydrophobic effect. Characterization of the dechlorination products is of interest since one can determine the effects of dechlorination upon lipophilicity and hence, possibly toxicity. For example, dechlorination of photodieldrin by this mechanism may be detoxifying in view of the drastic change in lipophilicity as measured by gas chromatography. More recent studies are using anionic surfactants as possibly more effective catalysts since there is data supporting an ionic mechanism, possibly carbonium ion in nature. In addition, recent studies are examining the effects of micellar systems on the photolytic reactions of the polycyclodiene pesticides as initial model chlorinated hydrocarbon systems.

Other studies utilizing reporter molecules and lanthanide NMR shift reagents have examined the behavior of the reversed micellar system of lecithin in benzene in the presence of water. As a result of their remarkable cooperative solvent solubilizing effects, lecithin reversed micelles aggregate polar, lipophilic compounds such as bis(p-chlorophenyl) acetic acid (DDA) and kelthane to an extent that electrostatic interactions of an intermolecular type may be operative. It is certain that the microenvironment of the solubilized reporter molecule has changed and that such changes in the stereoelectronic nature of the molecule could elicit unique chemistry. This solubilizing ability of the lecithin system was lost on formation of the carboxylate anion of DDA in the polar head group.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to gain understanding and insight into the possible human health effects of the chlorinated hydrocarbons, one needs to correlate hydrophobicity (interaction with biological membranal and cellular components) and biological potency, metabolism and biological potency, and biological potency with the overall symmetry and chemistry properties inherent to structure.

PROPOSED COURSE: In making the above correlations we hope to rank the various chlorinated hydrocarbon systems according to hydrophobicity (lipophilicity), propensity to undergo reductive dechlorination (oxidative dechlorination, hydrolysis and conjugation) leading to possible metabolites, and structural symmetry effects on micellar association and chemistry.

PUBLICATIONS

McKinney, J.D. and Palaszek, S.M. dePaul: Chemical reactions in micellar systems. Reductive dechlorination of some chlorinated polycyclodiene pesticide systems. For presentation at the Third International Congress of Pesticide Chemistry, Helsinki, Finland, July 1974.

Serial No.: NIEHS-EBC-022-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Hydrocarbons as Model Compounds for Metabolic Studies"

PREVIOUS SERIAL NUMBER: NIEHS-AST-042

PRINCIPAL INVESTIGATOR: Phillip W. Albro, Ph.D.

OTHER INVESTIGATORS: R. Thomas, Briant Moore

COOPERATING UNITS: None

MAN YEARS

Total:	0.25
Professional:	0.15
Other:	0.10

PROJECT DESCRIPTION

OBJECTIVES: To determine to what extent hydrocarbons may be used as model compounds in predicting metabolic modification of aliphatic chains in other classes of compounds.

METHODS EMPLOYED: The basic test organism is the rat, to which hydrocarbons are administered orally in corn oil, mixed with rat chow, injected I.P., I.V. or I.M. In some cases radioactive tracers (1-^{14}C-hexadecane, 1-^{14}C-octadecane, and U-^{14}C-phytane and 2,3-^{3}H-phytane synthesized here) are used in metabolic studies. Standard chromatographic, immunologic, radioassay and other biochemical techniques are used, except that balance studies of absorption from the gut are made as described in Biochim. Biophys. Acta 219: 437-446, 1970.

MAJOR FINDINGS: (1) The polychlorinated hydrocarbons hexachlorobenzene and hexachlorocyclohexane are efficiently absorbed from the intestines of rats at relatively low doses (<30 mg/Kg). Hexachlorobenzene becomes very inefficiently absorbed as the dosage increases above 30 mg/Kg. The different isomers of hexachlorocyclohexane are absorbed at different rates, the most toxic isomer also being the most readily absorbed. (2) In vivo studies on the metabolism of phytane have been completed and a manuscript is being prepared for publication. The mode of attack on the phytane skeleton is indicative of a previously unknown pathway for degradation of aliphatic chains.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Biological mechanisms for the modification and degradation of aromatic, alicyclic and

aliphatic moieties are far from adequately understood. An understanding of *in vivo* capabilities in these areas will simplify metabolic studies of many classes of compounds, and hopefully permit reliable predictions as to what types of metabolites might be expected upon ingestion of various new drugs, pesticides, food additives, etc.

PROPOSED COURSE: *In vitro* studies are planned in an attempt to identify early metabolites in the degradation of isoprenoid hydrocarbons, since we feel that the early metabolites may be related to the adverse effects of these hydrocarbons on the immunologic competence in animals. The mechanism of attack at points of chain-branching, the new degradative pathway mentioned above, will be evaluated as to its generality and applicability to classes of compounds of greater environmental interest than hydrocarbons.

Serial No.: NIEHS-EBC-023-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Transport and Metabolism of Phthalate Esters"

PREVIOUS SERIAL NUMBER: NIEHS-AST-043

PRINCIPAL INVESTIGATOR: P. W. Albro, Ph.D.

OTHER INVESTIGATORS: R. Thomas, Briant Moore, Ann Latimer

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To prepare purified standard labeled and unlabeled phthalate esters, e.g., DHP [di(2-ethylhexyl)phthalate] and DOP-(dioctyl phthalates); and to elaborate aspects of their transport, absorption, excretion and tissue residues following various routes of administration, e.g., oral, inhalation and i.p., in the rat.

METHODS EMPLOYED: Chromatography (TLC, column, GLC), spectrophotometry (I.R., U.V., visible), mass spectrometry and isotopic methods, standard enzymology techniques.

MAJOR FINDINGS: (1) Only those phthalate esters having some water solubility (dimethyl, dibutyl) are absorbed from the intestine of rats intact to any extent. (2) In general, phthalate diesters are hydrolyzed in the intestines to an alcohol and a phthalate monoester, both of which are absorbed. This applies to, at least, rats, mice, hamsters and guinea pigs. The absorbed monoester is metabolized by ω- and ω-1 oxidation to highly soluble urinary excretion products, but the phthalate ring is not modified. The same pattern of metabolism occurs with n-butyl, n-octyl, and 2-ethylhexyl phthalates. (3) Non-specific lipase from rat pancreas has been characterized as a micelle-requiring, anionic micelle-binding, general acid/base ester hydrolase. Many of its properties have been determined.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our studies have indicated that no more than traces of phthalate diester plasticizer

will be absorbed intact from the intestinal tract of several species of animals. Non-specific ester hydrolases of ubiquitous distribution should eliminate the majority of those traces, for the water-insoluble diesters. The phthalate-containing hycholytic products and their oxidative metabolites are rapidly excreted. Accordingly, the only significant hazard from orally ingested phthalates of this type, if indeed there is a hazard, would probably be associated with the alcohol hydrolysis product.

PROPOSED COURSE: The ability of the lung lipases to hydrolyze phthalate plasticizers presented as vapors or aerosols remains to be studied. We are presently studying the *in vivo* metabolism of 2-ethylhexanol in rats.

PUBLICATIONS

Albro, P.W., Thomas, R., and Fishbein, L.: Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr. 76: 321, 1973.

Albro, P.W., and Thomas, R.: Enzymatic hydrolysis of di(2-ethylhexyl) phthalate by lipases. Biochim. Biophys. Acta 360: 380, 1973.

Albro, P.W., and Latimer, A.D.: Pancreatic non-specific lipase: An enzyme highly specific for micelles. Biochemistry (ACS) (In press).

Serial No.: NIEHS-EBC-024
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Ester Hydrolases at the Environmental Interface"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Phillip W. Albro, Ph.D.

OTHER INVESTIGATORS: Ann Latimer, Briant Moore

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the capabilities of various tissue lipases and esterases as early mediators in the interaction of animals with xenobiotics containing ester linkages.

METHODS EMPLOYED: Most studies will involve the rat, but significant findings will be checked in mice, hamsters, guinea pigs and rabbits. Lung, gastrointestinal, and skin enzymes will be studied, using techniques generally described previously (Biochim. Biophys. Acta, 360: 380, 1973). Test substances will initially include: (1) esters of 2,4-D and 2,4,5-T, (2) phthalate plasticizers, (3) detergents such as Tween 20, Tween 80, (4) aromatic subrefacients such as methyl paraben, menthyl salicylate, (5) volatile solvents such as ethyl acetate, butyl acetate, (6) carbomates.

MAJOR FINDINGS: This is a new project and little effort has been put into it as yet. However, hydrolysis of isopropyl and isooctyl esters of 2,4-D and 2,4,5-T has been observed in preparations of rat pancreas non-specific lipase, while the lipolytic hydrolysis of phthalate diesters has been discussed under a separate project.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively little is known about non-specific lipase activity, since the enzyme(s) has(have) never been purified. Although a great deal is known about lung esterases, very little is known about lung lipases (hydrolases for insoluble esters). Virtually nothing is known about ester hydrolases in skin. These

enzymes may either protect against some hazardous compounds by converting them to less easily absorbed, more polar materials, or increase the hazard of other compounds when the acid (or alcohol) is more toxic than the ester. A second anticipated result of this project should be a better understanding of how enzymes in general are able to bind to and react with water-insoluble compounds.

PROPOSED COURSE: It is anticipated that one or more of the following studies will be begun shortly, depending upon the availability of personnel: (1) lung lipases, their substrate specificities and general kinetic properties, (2) skin lipases and esterases: do they exist? (3) an attempt to isolate pancreas non-specific lipase for eventual use as synthetic reagents.

Serial No.: NIEHS-EBC-025-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Biosynthesis of Piperidine and Pyrrolidine"

PREVIOUS SERIAL NUMBER: NIEHS-AST-045

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATORS: J. D. Peele, B. J. Corbett

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.1
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: Allyl benzene metabolic derivatives are linked biologically to tertiary amino propiophenones, e.g., the amine condensing units of each ketone are dimethylamine, piperidine and pyrrolidine. Very little information is available on the biosynthesis of these particular secondary amines. It is very desirable to determine the biological sequence of events that yield dimethylamine, and especially piperidine and pyrrolidine. Piperidine and pyrrolidine possess "nicotine-like" properties in biological systems. In addition, the correlation of psychoactive properties of allyl benzene metabolites of essential oil with that of these secondary amines should enhance the evaluation of potential hazard of these materials as environmental agents.

Because of the production of the carcinogenic nitrosamines of piperidine and pyrrolidine in foods and other biological systems, a thorough evaluation of these secondary amines is greatly needed.

METHODS EMPLOYED: Chromatography (TLC, column and GLC), cell fractionation by ultracentrifugation, enzyme analysis, and radioactive tracer metabolism followed by NMR, IR and mass spectrometry.

MAJOR FINDINGS: Methods of analysis of piperidine and pyrrolidine have been evaluated and developed for the quantitative and qualitative analysis of these secondary amines in biological systems including human urine and ratbrain. Dinitrophenyl derivatives of piperidine and pyrrolidine have been synthesized and characterized by TLC, GLC, NMR, IR, visible spectroscopy, and mass

spectrometry. Spectrophotometric analyses of the dinitrophenyl derivatives of piperidine and pyrrolidine indicate linearity over a range of 0.5 to 7 µg/ml. Recovery of exogeneous piperidine from human urine ranges from 26% to 42%. Recovery of exogeneous piperidine from rat brain supernatant ranges from 50-54%.

Preliminary investigations have indicated that the DNP amine can be detected in picogram quantities using gas chromatography with an electron capture detector. Because of the desirable detectable limits of DNP-piperidine (at least 10 picograms) by electron capture, this method of analysis appears most promising for quantitation of low concentrations of piperidine and pyrrolidine in biological tissues. In addition, this gas chromatographic analysis eliminates previously required TLC separation and elution, therefore, minimizing manipulations which greatly decreases the recovery of these amine derivatives

Because of the very low and non-signified decarboxylase activity as assayed by monometric assay with proline and pipecolic acid, investigations were initiated with carboxy labeled ^{14}C-pipecolic acid and proline to determine the significance of this decarboxylation to form piperidine and pyrrolidine respectively. Using rat brain, liver, and kidney, no detectable decarboxylase activity was obtained. Furthermore, chemical investigation indicated that upon steam distillation of pipecolic acid, piperidien is formed non enzymatically. From these investigations it seems that the biosynthesis of piperidine and pyrrolidine in mammals progress through an indirect pathway not by a simple direct decarboxylation of pipecolic acid and proline respectively.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding the knowldege of the rates of production and elimination of piperidine and pyrrolidine are essential in determining the potential of these biological materials as health hazards.

PROPOSED COURSE: (1) To investigate the formation of piperidine and pyrrolidine from other precursor including ^{14}C-glutamic acid and ^{14}C lysine. (2) To coordinate the biosynthesis of piperidine and pyrrolidine with the metabolic sequences leading to the formation of the tertiary amino propiophenons.

Serial No.: NIEHS-EBC-026-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolic Interaction of Amines and Tertiary Amino Propiophenones with Monoamine Oxidase Systems"

PREVIOUS SERIAL NUMBER: NIEHS-AST-047

PRINCIPAL INVESTIGATOR: V. S. Bangdiwala, Ph.D.

OTHER INVESTIGATOR: E. O. Oswald, Ph.D., P. W. Albro, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To characterize the interaction and inhibition of the nitrogen metabolites of the allyl benzenes - namely the tertiary amino propiophenones and specific amines with mitochondrial monoamine oxidase systems.

METHODS EMPLOYED: U.V.-Visible Spectroscopy, cellular fractionation by ultracentrifugation, electron microscopy, and standard enzyme analyses.

MAJOR FINDINGS: Comparative studies indicate that tissues from young rats (100-150g) provided a much more desirable source of monoamine oxidase than those of older rats (350-500g). The purity and recovery of mitochondria from tissue homogenate (1:10) was determined by analysis of the enzyme maker system - cytochrome C oxidase along with monoamine oxidase and by electron microscopy. Approximately 60-70% of the monoamine oxidase activity was located in the mitochondria with 30-40% of the enzyme activity residing in the nuclear fraction. Little or no monoamine oxidase activity was found in the final supernatant fraction.

Preliminary inhibition studies were made with piperidine, pyrrolidine, the piperidine metabolite (II) of elemicin and the pyrrolidine metabolite (III) of safrole. The amines and tertiary amine propiophenones of elemicin and safrole exhibited varying degrees of inhibition dependent upon the tissue and the specific substrate as assayed by manometric techniques. For example, piperidine and pyrrolidine with serotonin as substrate did not inhibit liver, brain or

kidney mitochondrial monoamine oxidase, on the contrary with tyramine as substrate piperidine inhibited mitochondrial monoamine oxidase preparations of all three tissues 40-100%. The tertiary amino propiophenone metabolite of elemicin II and safrole III both inhibited liver, brain, and kidney monomic oxidate system with both tyramins and serotamin as substrate at levels varying from 60-100% inhibition.

More recently using the radio label assay for monoamine oxidase with ^{14}C-benzylamine as substrate, piperidine, pyrrolidine, dimethylanine, and the safrole metabolites I, II and III - all inhibited competitively the mitochondrial enzyme preparations from rat liver. The characteristics and potential for inhibition were investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough understanding of the interaction of the tertiary aminopropiophenones and specific amines with mitochondrial monoamine oxidase systems would help to remove the existing ambiguities related with the psychoactive properties of various components of essential oils. This information would furnish a better means of evaluation of these environmental agents as potential health hazards.

From these investigations, it seems that the nitrogen containing metabolite of the essential oils - namely the tertiary amino propiophenones by inhibition of monoamine oxidase system may account for the psychoactive properties of the allyl and propenyl benzenes.

PROPOSED COURSE: (1) Complete inhibition studies with the tertiary amino propiophenones and specific amines. (2) Termination of this area of research because of the unavailability of personnel and decreased emphasis on natural product environmental agents.

Serial No.: NIEHS-EBC-027-?
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolism of Allyl and Propenyl Benzenes"

PREVIOUS SERIAL NUMBER: NIEHS-AST-046

PRINCIPAL INVESTIGATOR: E. O. Oswald, Ph.D.

OTHER INVESTIGATOR: B. Corbett, J. D. Peele

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To identify the various urinary metabolites of myristicin, safrole, isosafrole, elemicin, eugenol, and asarone, following administration by various routes to the rat and other biological test systems. (2) To investigate the general metabolism of these allyl and propenyl benzenes with radiolabeled substrates. (3) To coordinate this chemical and metabolic information so that one may determine the potential hazards produced by these natural product environmental agents.

METHODS EMPLOYED: Chromatography (TLC, column, and GLC), NMR, IR and mass spectroscopy, and standard radioisotopic analyses.

MAJOR FINDINGS: Following oral or intraperitoneal administration of myristicin, safrole, isosafrole, elemicin, eugenol, asarone (trans) or beta asarone (cis) to male rats, basic ninhydrin positive materials are excreted in the urine. There is an apparent requirement of a side chain double bond for the production of these materials with greater enhancement by the trans isomer than the cis.

Complete structural identification of the ninhydrin positive metabolites of safrole, myristicin, elemicin and eugenol indicates that the nitrogen containing metabolites are tertiary amino propiophenones. The amine substituents of these tertiary amine propiophenones excreted by the rat and guinea pig are: (I) dimethylamino, (II) piperidine, and (III) pyrrolidine. Depending upon the structure of the administered allylbenzene and the specific test species, varying abundances of these specific amine substituted tertiary aminopropio-

phenones were noted. The piperidine substituted metabolites seem to be the major urinary nitrogenous metabolite of the allyl benzenes examined to date.

Initial metabolic investigations with methylenedioxy ^{14}C labeled safrole indicate that approximately 50% of the orally administered ^{14}C is released as $^{14}CO_2$ within 27 hours in the rat. Feces and urine account for about 20% of the label during the 27 hour period. Analysis of biological samples at varying time intervals indicates that the distribution of ^{14}C derived from methylenedioxy ^{14}C labeled safrole was general but not equally distributed throughout the rat. The liver contained the greatest amount of radioactivity for all time periods with the small intestine containing the second most concentrated quantity of label. The radiolabel from ^{14}C methylenedioxy safrole remaining in the liver seems to be associated with the ^{14}C-formate pool not with the original ^{14}C-allyl benzene.

Investigations with 3'-^{14}C-safrole indicate that the major quantity (80-90%) of label is excreted in the urine. No significant quantity (<5%) the labeled derived from the side chain was found in expired CO_2. About 1% of the label ^{14}C from 3'-^{14}C-safrole remained in tissues of the rat 48 hours after administration.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Unequivocal identification of the metabolites and the knowledge of the rates of elimination and degree of storage of these environmental agents are essential in determining the potential health hazards produced by exposure and injection of the allyl and propenyl benzenes and related compounds.

PROPOSED COURSE: (a) To complete the investigation on the ninhydrin positive metabolite of the allyl benzene using ring labeled and side chain ^{14}C labeled safrole, myristicin and elemicin in whole animal and cell free systems.

Serial No.: NIEHS-EBC-028-3
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Evaluation of a Captive-Born and Reared Colony of Opossums"

PREVIOUS SERIAL NUMBER: NIEHS-AST-014-2

PRINCIPAL INVESTIGATOR: Donald B. Feldman, D.V.M.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.1
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the physiologic, pathologic and reproductive parameters of a "closed" colony of captive-born opossums.

METHODS EMPLOYED: Opossums born during the 1973 breeding season (January - May) were caged individually in a "closed" facility. This colony was the third generation of captive-born young and consisted of 23 females and 16 males.

These opossums were free of endoparasites as was the previous captive-born generation. With the onset of the breeding season, Jan. 1, 1974, partitions separating 2 females and 1 male opossum were removed and the animals permitted to breed at will. Females were examined daily for the presence of young in the pouch and records of births were maintained.

MAJOR FINDINGS: The first litter was born Jan. 17, 1974. Thus far through 7 weeks of the breeding season the conception rate among captive-born opossums is 39%. This is a considerable improvement over the conception rate of 29% for the total (Jan. - June) 1973 breeding season. The addition of a calcium supplement to the diet may account for improved reproductivity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Maintenance of a closed breeding colony of captive-born opossums is a feasible means of providing sufficient animals for research from year to year. In addition, since they are born in captivity, they seem able to adapt to confinement better than a wild-captive animal.

PROPOSED COURSE: Maintenance and continued monitoring of a closed opossum colony if research demands are sustained. Diminished utilization of opossums at NIEHS may warrant complete or partial dissolution of the colony.

Serial No.: NIEHS-EBC-029-3
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Erythrocyte life-span in the Virginia Opossum (Didelphis marsupialis virginiana)"

PREVIOUS SERIAL NUMBER: NIEHS-AST-015-2

PRINCIPAL INVESTIGATOR: Joseph G. Zinkl. D.V.M., Ph.D.

OTHER INVESTIGATORS: Donald B. Feldman, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total: 0.1
Professional: 0.1
Other:

PROJECT DESCRIPTION

OBJECTIVES: To determine the erythrocyte life-span in the opossum, since many nucleated RBC's and many reticulocytes are seen in normal opossum blood smears.

METHODS EMPLOYED: Six 1.5 year old captive-born opossums were given 50 μCi glycine-2-^{14}C intravenously. The ^{14}C is incorporated into heme and eventually hemoglobin. Since the porphyrin moity of heme is not reutilized by the animal but rather is excreted as bilirubin, a measure of the time radioactive heme is found in erythrocytes determines their life-span. Therefore, heme was isolated, combusted to CO_2 and counted.

MAJOR FINDINGS: The erythrocyte life-span of the American opossum (Didelphis marsupialis virginiana) was determined by ^{14}C tagging of the heme moiety of hemoglobin using glycine-2-^{14}C to be 77 days. The survival time ranged from 71-82 days.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings suggest that the reason nucleated erythrocytes and a high reticulocyte count are found in peripheral blood smears of opossums is due to the need to replace these short lived erythrocytes. The animal responds by releasing immature cells from its bone marrow in order to maintain its blood's oxygen carrying capacities.

PROPOSED COURSE: The project is completed.

PUBLICATIONS

Zinkl, J.G., Feldman, D.B.: The erythrocyte life-span in the American opossum (*Didelphis marsupialis virginiana*). Lab. Anim. Sci. (In Press).

Serial No.: NIEHS-EBC-030-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Prostaglandins as Environmental Factors"

PREVIOUS SERIAL NUMBER: NIEHS-AST-051

PRINCIPAL INVESTIGATORS: Phillip W. Albro, Ph.D.

OTHER INVESTIGATORS: Richard Thomas, Priscilla Beckwith

COOPERATING UNITS: None

MAN YEARS

Total:	0.25
Professional:	0.0
Other:	0.25

PROJECT DESCRIPTION

OBJECTIVES: (1) Development of a highly sensitive, specific analytical procedure for qualitative and quantitative determination of prostaglandins. (2) Evaluation as to whether exogenous prostaglandins should be considered environmental health factors, either relative to ingestion or topical exposure. (3) Determination as to whether endogenous prostaglandins play a significant role in the response of animals to drugs, pesticides, antigens and the like.

METHODS EMPLOYED: Have been described in two previous publications (J. Chromatogr. 44: 443, 1969; Prostaglandins 1: 133, 1972).

MAJOR FINDINGS: The only effort put into this project this year was the institution of a library search for material relating prostaglandins to allergy and inflammation. This was not completed due to expiration of Miss Beckwith's term of appointment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Having eliminated several other possibilities, we have presently concentrated on the role of prostaglandins in the anaphylactic response as being related to their possible environmental significance and not under saturating study elsewhere.

PROPOSED COURSE: Upjohn has begun to license prostaglandins for drug use in this country. Should reason to believe harmful effects may arise from such use appears in the future, we may consider reinstituting this project on an active basis; but in view of the many types of negative findings we have experienced

to date, we anticipate that this project will be officially terminated upon completion of the literature review mentioned above.

Serial No.: NIEHS-EBC-031
1. Environmental Biology and Chemistry Branch
2. Comparative Biology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Aerosol Hair Spray Inhalation Effect"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Bhola N. Gupta, B.V.Sc.& A.H., Ph.D.

OTHER INVESTIGATOR: Robert T. Drew, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.1
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: To determine the inhalation effect of aerosol hair spray in the hamster.

METHODS EMPLOYED: Hamsters were exposed to a commercially available aerosol hair spray for a total of 8 minutes in a 4-hour-period/day 5 days/week. They were examined after 1, 2, 3, 4, and 6 week exposure, and 2, 4 and 8 weeks after a 6-week exposure.

MAJOR FINDINGS: There was a significant increase in the lung weight of exposed hamsters at every time point except 8 weeks after the 6-week exposure. Microscopic changes in the lung were characterized by the presence of a few to moderate number of macrophages and heterophils in the alveolar lumina. The magnitude of the presence of macrophages and heterophils appeared directly related to the duration of exposure. Lung changes were minimal after the first 2-weeks of exposure and 8 weeks after the final exposure. Pathologic changes in the lungs of hamsters were reversible after removal of the hair spray inhalation exposure. Inhalation of the aerosol hair spray appears to be slightly irritating to the lung of hamsters.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many cosmetics and other products are readily available in aerosolized forms. Aerosol cans are ideally suited for topical application and their efficiency and convenience are undisputed. Small aerosol droplets could be inhaled while

spraying. Although reversible, slight irritating effects were observed in the lungs of hamsters after inhalation of aerosol hair sprays.

PROPOSED COURSE: Project completed.

Serial No.: NIEHS-EBC-032-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "^{13}C NMR Studies of Binding in Aromatic Carcinogens"

PREVIOUS SERIAL NUMBER: NIEHS-AST-049

PRINCIPAL INVESTIGATORS: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS:

COOPERATING UNITS:

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To obtain and analyze the ^{13}C NMR spectra of polynuclear aromatic carcinogens, to investigate the effect of possible complexing agents on these spectra and thus illuminate the possible modes of interaction of the aromatic carcinogens in biological systems.

METHODS EMPLOYED: Variable temperature nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: ^{13}C NMR data for model systems for the carcinogenic-methylated polynuclear aryl hydrocarbons, the methylated naphthalenes and methylated anthracenes, have been obtained to aid in analysis of the carcinogen data. Preliminary studies of the binding propensities of the carcinogens have begun.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although work on carcinogenic compounds of this type has been extensive, and the hazards associated with their production and use are well-documented, their mode of interaction in biological systems is not fully understood. The studies being conducted here may help to elucidate the mechanisms of carcinogenicity of these compounds.

PROPOSED COURSE: Completion of this project within the next few months is anticipated.

PUBLICATIONS

Wilson, N.K. and Stothers, J.B.: ^{13}C and ^{1}H spectra of some methyl-naphthalenes. J. Magnetic Resonance 15: 1974 (In Press).

Serial No.: NIEHS-EBC-033-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "NMR Investigations of Metallic Linear and Cyclic Dithiocarbamates"

PREVIOUS SERIAL NUMBER: NIEHS-AST-050

PRINCIPAL INVESTIGATORS: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine activation and thermodynamic parameters for the decomposition of a variety of cyclic and linear metallodithiocarbamates and to investigate the molecular and electronic structures of these compounds.

METHODS EMPLOYED: Variable temperature nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: Preliminary variable temperature NMR data has been obtained.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The cyclic and linear metallodithiocarbamates have wide utility as fungicides and rubber accelerators and, hence, are of environmental significance. Ethyl selenac has also been shown to be an effective enzyme inhibitor. Elaboration of the molecular structures of these compounds, possibly decomposition products and their rates of formation may illuminate both the mode of inhibition and the possible environmental hazards associated with the use of dithiocarbamates.

PROPOSED COURSE: Termination of this project within the next few months is anticipated.

Serial No.: NIEHS-EBC-034-2
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "NMR Investigations of Structures and Molecular Interactions of Nitrosamines"

PREVIOUS SERIAL NUMBER: NIEHS-AST-048

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the detailed molecular geometry and electronic structures of aliphatic and aromatic nitrosamines; to study their molecular interactions with models for biologically significant systems, and to determine the possible means of their formation and decomposition in such model systems.

METHODS EMPLOYED: Variable temperature nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: Variable temperature ^{13}C NMR data have been obtained for several simple nitrosamines.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nitrosamines are known potent carcinogens; they have been found to occur in certain foods, and additionally, they may possibly be formed in the gastro-intestinal tract from nitrites and nitrates in foods. Thus knowledge of their molecular properties and chemistry is of great importance in evaluation of the present and potential hazards due to this class of compounds.

PROPOSED COURSE: Termination of this project within the next few months is anticipated.

Serial No.: NIEHS-EBC-035
1. Environmental Biology and Chemistry Branch
2. Chemistry Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "NMR Investigations of Organomercury Compounds"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Nancy K. Wilson, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the detailed molecular geometrics, electronic structures, and possible molecular interactions with biologically significant systems, of several organomercury compounds.

METHODS EMPLOYED: Nuclear magnetic resonance spectroscopy.

MAJOR FINDINGS: ^{13}C NMR data have been obtained for a series of alkylmercury halides and for a series of substituted phenylmercury chlorides.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ubiquitous presence of mercury compounds and particularly alkylmercury compounds in the environment is well-established. Health hazards resulting from this research relates bonding and binding propensities to molecular structure and has value in prediction of possible future hazards as well as interpretation of these already established.

PROPOSED COURSE: Completion of this project within the next few months is anticipated.

ENVIRONMENTAL BIOMETRY BRANCH

ENVIRONMENTAL BIOMETRY BRANCH
Summary Statement

The Environmental Biometry Branch fulfills a dual mission, functioning both in a service capacity to the intramural program, and also as an applied research group. Statistical support for the Institute is provided mainly for the design of experiments and in the analysis of laboratory data. Data processing and computer programming services are available to the research staff of the Institute and are routinely employed by the Environmental Biometry Branch in both its statistical applications and research work.

Research activity is currently being conducted in the general areas of statistical methodology, biomathematics and epidemiology. The statistical research involves both the development of methodology generated from data analysis needs, and the investigation of topics such as clinical trial design and low-dose extrapolation, which are of abiding interest to NIH. Biomathematics research is primarily collaborative, being focused mainly on the mathematical modeling of biological processes. Finally, the epidemiological work being done is concerned with the statistical analysis of existing data bases, the emphasis being on an attempt to detect environmental components and quantify their implications upon human health.

The following breakdown gives and indication of the specific areas within the intramural program where statistical applications and data processing services are rendered.

STATISTICAL AND PROGRAMMING SUPPORT

ENVIRONMENTAL TOXICOLOGY BRANCH

Environmental design and statistical analyses were provided to the Environmental Toxicology Branch for a study investigating the feasibility of assessing the teratogenic potential of a compound by direct intrauterine injection. Fetuses in one horn receive the compound; fetuses in the other horn, a sham exposure. Thus, pairwise comparison techniques were employed to determine the significance of treatment-control differences, in conjunction with auxiliary tests for possible carryover effects. The usefulness of this procedure was evaluated for thalidomide, a known teratogen.

Response surface techniques were used to estimate levels of DMSO, holding time, cooling and warming rate and terminal temperature that maximized in vitro development of rabbit morulla following an 18 hour freezing period. The experimental design consisted of a series of complete or fractional experiments, each utilizing 1-3 levels of the five factors. Other potential sources of variability, such as differences among embryo pools, were also examined.

In a related study, statistical analyses were performed to determine the effect of estrogen, progesterone, and prostoglandis on in vitro blastocoele development.

Because of the large volume of teratology data being generated at NIEHS, it is desirable to have a computing system capable of providing summary information and some routine statistical analysis for this type of data. While there is a great deal of variability in the experimental protocols employed by various scientists, efforts are continuing to develop a system that, with some modifications, can satisfy a variety of individual needs.

In order to assess the effects of heavy metal toxicity on learning in invertebrates, three groups of shrimp were conditioned by shock stimulus over a 21 day interval to avoid a randomly flashing electric light. One group was exposed daily to methyl mercury concurrently with conditioning, another was exposed following conditioning, and a third was unexposed. Multiple comparisons of the proportion of correct responses exhibited by each group were made on both a daily and an overall basis.

Experimental design and data analysis services were provided to assist in assessing the effects of methyl mercury exposure on shell selection instincts in hermit crabs. Effects investigated included species response differences and interaction of environmental stimuli with shell selection mechanisms.

The experimental design for a long-term breeding study initiated to investigate the effects of diethylstilbestrol on oogenesis was developed. Subsequent to the initiation of the study modifications in the design allowing for removal of selected treated females (for use in concomitant investigations) in a manner that would yield unbiased tests and estimates of various responses at the conclusion of the study were explored. Data from a similar study based on pre-exposure to DDT was also analyzed. Particular emphasis was given to the inner-relationship between number of litters produced and average litter size.

Statistical analyses were performed for several assays studying the dominant lethal and anti-fertility effect of certain environmental chemicals and anti-cancer drugs in mice. Of particular interest in these studies was the determination of the stage of spermatogenesis most susceptible to the agents' adverse effects. Nonparametric tests were applied to determine the significance of treatment-control differences in live fetuses, implants, pregnancy rates and other parameters.

Multiple comparison procedures assuming an underlying monotonic response were utilized to determine the shortest pre-exposure (to 85% oxygen) time interval required to significantly elevate lung SOD levels in rates exposed to 100% pure oxygen. In a related experiment, estimates of the ratios of treatment-control SOD levels along with their approximate variances were derived to produce a graphical representation of the pre-exposure phenomenon free of confounding experimental replicate effects.

Data was analyzed from a study investigating the effect of maternal thyroparatyyroidectomy on fetal survival and development as well as the possible alteration of this response by the addition of thyroxine or parathyroid hormone. Intercorrelations among the variables were also examined in order to gain possible insight as to the underlying mechanisms involved.

In a multi-factorial experiment analysis of variance procedures were employed to determine how blood vessel constriction in rats was affected by cadmium, low dietary calcium, length of treatment and blood vessel diameter. Certain main effects and second-order interactions were found to be major sources of variability.

PHARMACOLOGY BRANCH

Extensive statistical support was provided for a multiphase study of the effect of phenobarbital on chronic benzene toxicity in rats. A spectrum of indices of toxicologic response was investigated, including lung and liver rates of metabolism, body and organ weight change, and variations in a variety of hematologic parameters. All phases of the experimental design were reviewed in order to identify the most appropriate measures of response and to delineate major sources of variability. Because of the small sample sizes involved in many of the comparisons, nonparametric procedures incorporating experiment-wide error rates were employed whenever possible.

Consulting and data analysis services were given to assist in the characterization of charoxyhemaglobin production and decay in rabbits following inhalation of dichloromethane. Multiple comparison procedures were used to evaluate the effects of various metabolic inducers and regression techniques were employed to quantify changes in levels of carboxyhemaglobin.

Non-linear regression capabilities were developed and applied to various data from enzyme kinetic and multi-compartment distribution models. Included were various multi-exponential models. Data analyzed typically concerned enzymatic processes in isolated perfused lungs.

Statistical support was provided for a study of the effects of known inducers of drug metabolizing enzymes on in vitro benzene metabolism following different routes of administration. Nonparametric techniques based on a direct extention of the Spearman-Karber procedure were employed to estimate and compare treatment-control differences in LC_{50} and LD_{50} values. Route dependent responses were delineated.

Data was analyzed from an investigation of the effects of inhaled fluorocarbons on the cardiovascular parameters of rabbits. Effects of several specific compounds were evaluated and compared, and dose-response relationships were assessed where appropriate.

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH

Statistical support was provided to the Environmental Biology and Chemistry Branch for several related studies investigating the adverse effect of dioxin on laboratory animals. In one study it was desired to determine the effect of dioxin on the cell medicated immune system in rats and mice under a variety of experimental conditions. A second study investigated the effect of dioxin on mammary gland DNA and RNA. A third study was interested primarily in organ and body weight changes. In all, these studies of treatment-control comparisons were made and nonparametric techniques applied to test for overall dose-response relationships.

Nested analysis of variance procedures were employed to assess the magnitude of treatment-control differences in hamster body weight at different points in time following inhalation chamber exposure to hairspray. The effect of the hairspray on various organ weights was also examined.

In order to determine the potential toxicity of low levels of PCB administered in the diet overall trends as well as individual treatment-control differences at specific points in time were evaluated using various nonparametric procedures. Various statistical procedures for measuring the effect of starvation at the conclusion of the feeding study on blood chemistry are currently being examined.

ENVIRONMENTAL MUTAGENESIS BRANCH

As part of the evaluation of a series of experiments designed to investigate potential genetic variability associated with heavy metal sensitivity, the relative frequencies of various allozmic phenotypes among control and treated (with a variety of heavy metals) populations of flies were compared. Experience gained from this analysis will be incorporated into a computer program that is being developed to handle large data sets arising from similar screening studies.

Small measurement errors of individual dilutions can result in dramatic increases in the variability of estimated initial particle concentrations. Since this variability is usually estimated without taking into account possible dilution errors, the investigator will falsely believe his experimental precision to be lower than it actually is thus explaining situations of apparent lack of reproducibility. The statistical effects of these small dilution errors have been determined when serial dilutions are required. Also, optimal dilution designs have been developed.

Data were analyzed and theoretical models weee investigated for experiments on induced mutation. The data were found to be consistent with periodicities with increased amplitudes as a function of time of exposure. Theoretical "hit" models explaining the periodicities were examined.

ENVIRONMENTAL BIOPHYSICS BRANCH

In a study investigating noise-induced ototoxicity in guinea pigs, multiple comparison procedures were employed to determine the relative susceptibility of newborn, young and adult animals to cell damage in the organ of corti following identical noise exposures. Treatment-control comparisons at each age were also made to assess the magnitude of the damage.

Quail chick blood chemistry data were analyzed to determine the effects of microwave exposure of the embryos. Numerous blood parameters were examined, with exposures occurring at several different stages of embryonic development. Treatment-control differences were assessed and associations of time of exposure with blood abnormalities were sought.

OFFICE OF THE DIRECTOR

As an outgrowth of a meeting of various pediatrics groups supported by the NIEHS "Alert Physicians" program called to review data on the possible correlation

between the use of progestational drugs in diagnostic pregnancy tests and the subsequent onset of infant CHD, the Biometry Branch was asked to comment on several past studies dealing with this issue and to evaluate a proposed prospective investigation in the same area. The requested review indicated that some of the past studies were inconclusive at best, and sample size simulations based on estimates from these studies raised doubts about the ability of the proposed investigation to detect increases in TGV disorders even if they were associated with drug usage.

Data was analyzed from an inhalation study investigating the rats respiratory tract susceptibility to the putative carcinogenic effects of nickel sulfide. A secondary objective was to determine whether an enhancing effect could be induced by pre-treatment with an agent causing pulmonary infarction. After adjusting for survival differences, treatment-control comparisons were made for tumor incidence and epithelial cell proliferations in the lungs. Possible adverse effects on other organs were also considered.

RESEARCH ACTIVITIES

STATISTICAL METHODOLOGY

Methods for the extrapolation of experimental results to low dose levels are being investigated. Two models, Mantel-Bryan and Albert-Altshuler, are being compared. For these models, questions concerning sensitivity and robustness are of particular interest.

Statistical techniques are being studied for the problem of determining the lowest dose which produces a significant effect compared to the control. Results have been obtained for normally distributed response variables and work is continuing on the problem of dichotomous response variables.

The development of efficient clinical trial designs is being continued. Designs which select the treatment with the best 5 year survival rate have been developed. Likelihood biased sampling has also been applied to these designs.

MATHEMATICAL BIOLOGY

In collaboration with the Pharmacology Branch research efforts in pharmacokinetics were concentrated in two areas: 1) investigation of the uptake, accumulation, metabolism and release of chemicals by the lung using the isolated perfused lung system, and 2) development of pharmacokinetic models for several of the polychlorinated biphenyls (PCB).

An isolated perfused lung system was developed so that initial rates of uptake and efflux of accumulated chemicals could be investigated. Using this system, detailed models for the dynamic interaction between the lung and several basic amines have been developed. For the exogenous amine, imipramine and methadone transport system is not involved in their uptake since the initial velocity of uptake is linearly related to concentration. Efflux studies show that several types of binding sites are responsible for the accumulation of these amines by the lung. These studies also showed that if the amine is allowed to accumulate, then some of it cannot be effluxed. This non-effluxable component of accumulated

methadone or imipramine is being pursued in more detail. Although it has been previously demonstrated that 5-HT is detoxified by the lung, the system has permitted the first characterization of the dynamic aspects of the transport and the metabolic component of this system and of the interactions between them. The vasoactive hormone is removed from the circulation by a uni-transport system, metabolized by the intracellular monoamine oxidase to 5-hydroxy indole acetic acid and then the metabolic effluxes by diffusion back into the circulation. Mathematical models are being developed which will allow the prediction of the dynamics of the accumulation of these amines by the lung for any given blood concentration. The methodology and techniques that have been developed can be used to study these aspects of lung interactions with any chemical.

The development of pharmacokinetic models for several PCBs is being attempted. In particular, feasibility of developing pharmacokinetic models is being investigated for these very lipophilic compounds similar to those constructed for several drugs by workers at NCI (Dedrick, Birchoff, Zarharbo, et al). These models are constructed from basic biochemical and physiological parameters. Analysis of time-distribution curves for the PCBs show that the liver plays a very important role in determining the distribution, metabolism and excretion into bile can be examined. This information will be used in developing the model. Other than the liver, the other important storage sites for the PCBs are muscle, fat and skin. The concentration ratio of the PCBs between muscle and blood remains essentially constant 10 minutes after an i.v. dose. Thus, the mathematical equation for the muscle component is straightforward. At present, equations are being developed which will predict fat and skin levels if the blood concentrations are known as a function of time. The only parameters in these equations are the blood flow rates and a partition coefficient of the PCB between fat or skin and blood. After these various studies are completed, there will be available a pharmacokinetic model for the PCBs or an understanding why this type of model (Dedrick et al) cannot be developed for these compounds.

EPIDEMIOLOGY

A cohort of 204 psoriasis patients who had been treated with methotrexate between 1960 and 1965 were followed over a seven year interval, and their observed mortality and malignancy rates were compared to simulated expected values based on survey data. Hepatic-related deaths seemed to be elevated, but the incidence of overall malignancy in terms of either mortality or tumor onset did not appeal to be markedly increased. A case-control prospective study is undoubtedly needed to obtain a definitive evaluation of the possible effect of methotrexate treatment on morbidity and mortality.

When data from certified death certificates occurring in North Carolina between 1946 and 1966 were analyzed using Knox's test criteria, no significant evidence of time-space clustering among fetal deaths attributable to either anencephaly or spina bifidia was detected. These findings concur with those reported in two previous studies (in Quebec and Providence, R.I.) and tend to argue against an infectious hypothesis.

Data from a large epidemiology study was analyzed to determine if the prevalence of corneal arcus was correlated with cholesterol levels, blood pressure, on

ponderal index, or was predictive of subsequent coronary heart disease. Race, sex and age differences in the prevalence rate of corneal arcus were also examined.

Serial No.: NIEHS-B-001
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Clinical Trials and Statistical Selection Procedures"

PREVIOUS SERIAL NUMBER: NIEHS-B-005

PRINCIPAL INVESTIGATORS: D. G. Hoel, Ph.D. and G. H. Weiss, Ph.D. (DCRT)

OTHER INVESTIGATORS: None

COOPERATING UNITS: none

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop efficient experimental designs for selecting the best of several experimental treatments.

METHODS EMPLOYED: Data from controlled laboratory or clinical trials are assumed to occur sequentially in time where the individual responses are dichotomous. Sequential regions have been developed for deciding when to terminate the experiment and which treatment to select as most effective.

MAJOR FINDINGS: New and improved procedures are continuing to be studied. Designs which select the treatment with the best 5 year survival rate have been developed and compared. Likelihood biased sampling has also been applied to these designs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The emphasis of these sequential designs is to minimize the number of trials on inferior treatments. This has been overlooked in classical clinical trials and is a critical concern when dealing with human populations.

PROPOSED COURSE: Continued improvement in the efficient use of laboratory and clinical trial data is required. Procedures are being studied which assume that the experimenter is in a treatment versus control framework. These procedures will differ from the ones obtained from the classical selection procedures which determine the best population.

PUBLICATIONS

Hoel, D. G. and Weiss, G. H.: Comparison of methods for choosing the better of two negative exponential lifetime distributions. In Proschan, Frank and Serfling, R. J. (Eds.): Reliability and Biometry. Philadelphia, SIAM, 1974, pp. 563-583.

Hoel, D. G., Sobel, M. and Weiss, G. H.: A survey of adaptive sampling for clinical trials. Biometrics (to appear)

Hoel, D. G., Sobel, M. and Weiss, G. H.: Comparison of sampling methods for choosing the best binomial population with delayed observations. J. Statist. Comp. and Simul. (to appear)

Serial No.: NIEHS-B-002
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Mathematical Models for Estimating Mutation Rates in Cell Populations"

PREVIOUS SERIAL NUMBER: NIEHS-B-019

PRINCIPAL INVESTIGATORS: D. G. Hoel, Ph.D. and K. S. Crump, Ph.D. (Louisiana Tech University)

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.1
Professional:	0.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To construct probabilistic models for describing the number of mutant cells in a cell colony as a function of time, and to develop estimates of cell mutation rates for the various models while taking into account experimental laboratory procedures.

METHODS EMPLOYED: Both Markovian and various types of deterministic growth were assumed for mutant and non-mutant cells. Mutants were assumed to be generated as a Poisson process. From these assumptions, the number of mutant and non-mutant cells may be expressed as functions of time.

MAJOR FINDINGS: Methods for estimating the cell mutation rate have been developed based on the models for cell growth. These estimates take into account laboratory procedures for periodic sampling of the cell cultures. Also, the effects of phenotypic delay have been studied and methods for adjusting the mutation rate estimates have been developed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is necessary both to develop better and more realistic methods for estimating cell mutation rates and to understand the effects of the mathematical assumptions which are a basis for mutation rate estimation.

PROPOSED COURSE: To continue to improve mutation rate estimation and to develop estimates for the diploid locus case.

PUBLICATIONS

Hoel, D. G. and Crump, K. S.: Estimating the generation-time distribution of an age-dependent branching process. Biometrics 30: 125-136, 1974.

Crump, K. S. and Hoel, D. G.: Mathematical models for estimating mutation rates in cell populations. Biometrika (in press).

Serial No.: NIEHS-B-003
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Probability Models and Competing Risks in the Study of Mortality Data"

PREVIOUS SERIAL NUMBER: NIEHS-B-006

PRINCIPAL INVESTIGATOR: D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: N. Chand, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop statistical techniques for assessing the effects of a treatment on specific diseases in survival studies.

METHODS EMPLOYED: Probabilistic models are being studied for describing the occurrences of particular diseases in a competing risk framework. Specific parametric models are being considered for low-dose extrapolation.

MAJOR FINDINGS: Models which are essentially equivalent in the experimental range may lead to drastically different results at low-dose levels. Therefore, careful attention must be given to all model assumptions.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The extrapolation of experimental laboratory results to low-dose human effects is a major environmental problem. These methods will be incorporated into the policy of setting "safe-levels" of environmental contaminants.

PROPOSED COURSE: Continued research into the sensitivity and robustness of low-dose extrapolation methods.

PUBLICATIONS

Chand, N. and Hoel, D. G.: A comparison of models for determining safe levels of environmental agents. In Proschan, Frank and Serfling, R. J. (Eds.): Reliability and Biometry, Philadelphia, SIAM, 1974, pp. 382-401.

Serial No.: NIEHS-B-004
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Statistical Methodology and Modeling in the Analysis of Teratology Data"

PEEVIOUS SERIAL NUMBER: Same

PRINCIPAL INVESTIGATOR: J. K. Haseman, Ph.D.

OTHER INVESTIGATORS: T. A. Clemmer, M.A. and M. J. L. Carver, M.A.T.

COOPERATING UNITS: None

MAN YEARS

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop a computing system for the statistical analysis of teratology data.

METHODS EMPLOYED: Computer programs are being written for the processing of data from several different types of teratology studies. Statistical subroutines are being developed to calculate summary statistics, to determine the significance of treatment-control differences and to examine overall dose-response relationships.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The large volume of teratology data currently being generated by various NIEHS studies has necessitated the development of a computerized system for statistical analysis. Such a system will greatly facilitate routine data processing.

PROPOSED COURSE: To continue to develop new statistical subroutines as the need arises and to increase the flexibility of existing programs in order to satisfy the individual needs of various scientists.

Serial No.: NIEHS-B-005
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Testing for Ordered Alternatives with Increased Sample Size for the Control"

PREVIOUS SERIAL NUMBER: None

PRINICPAL INVESTIGATOR: G. R. Chase, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To develop statistical tests that have greater power than those currently in the literature for assessing a dose-response relationship when the control sample size is larger than that employed at the various dose levels.

METHODS EMPLOYED: Following a thorough literature search, theoretical and Monte Carlo investigations were made to investigate possible tests.

MAJOR FINDINGS: When all sample sizes are equal, tests due to Bartholomew are the most powerful for a broad range of alternatives. The theory for the case of an infinite sample size for the control for Bartholomew's tests has been developed. Using these results, a very good approximation, supported by Monte Carlo results, for intermediate sample sizes has been found. Tables for the critical values have been compiled.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Increased sample sizes for control doses are often used when several levels of a treatment are investigated simultaneously. The tests will provide increased statistical power to these investigations.

PROPOSED COURSE: The project will be terminated.

Serial No.: NIEHS-B-006
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Investigation of Potential Time-Space Clustering on Neural Tube Defects in a Human Population"

PREVIOUS SERIAL NUMBER: NIEHS-B-017

PRINCIPAL INVESTIGATORS: M. D. Hogan, Ph.D. and D. G. Hoel, Ph.D.

OTHER INVESTIGATORS: M. J. L. Carver, M.A.T.

COOPERATING UNITS: North Carolina State Board of Health

MAN YEARS

Total:	0.3
Professional:	0.2
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To investigate fetal deaths attributable to anencephaly and spina bifida occurring in North Carolina between 1946 and 1966 for any evidence of time-space clustering among cases.

METHODS EMPLOYED: Fetal death certificates with an underlying cause of anencephaly or spina bifida were reviewed, and estimates of the time of conception and longitudinal and latitudinal co-ordinates of the mother's residence were obtained. The data was divided into three seven-year intervals and all possible pairs of cases were examined for clustering within various predetermined time-space grids using Knox's test criteria.

MAJOR FINDINGS: No evidence of significant time-space clustering was found for either type of fetal death for any of the time periods under investigation. While some indication of clustering among anencephalic infant deaths was noted during the last time period, it disappeared when fetal and infant deaths were combined and reanalyzed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study supports previous investigations in Quebec and Rhode Island which argue against an infectious agent having a major rule in the etiology of these CNS disorders.

PROPOSED COURSE: (1) Urban-rural differences in monthly and seasonal incidences of fetal deaths attributable to anencephaly and spina bifida are being examined for any evidence of association with estimated pesticide usage. (2) The possibility of an environmental agent acting on the mother during her prepuberital years and effecting her subsequent pregnancy history is also being investigated.

Serial No.: NIEHS-B-007
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Nonparametric Tests in Biometry"

PREVIOUS SERIAL NUMBER: NIEHS-B-014

PRINCIPAL INVESTIGATORS: K. M. Patel, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To develop a generalized Friedman test for (comparing several treatments in) a randomized block design when responses are subject to arbitrary right censorship. (2) To develop, along the lines of Govindarajulu (1968), a distribution-free or nonparametric confidence ellipsoid (based on two independent random samples of multidimensional responses) for comparing two treatments.

MAJOR FINDINGS: (1) A generalized Friedman test for comparing several treatments in a randomized block layout subject to (possibly unequal) patterns of censorship from block to block has been developed. (2) An approximate nonparametric confidence ellipsoid indicating the differences in component effects when the underlying responses are continuous is being investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: (1) A clinical trial or survival study in which the effects of several treatments are compared may be designed in a randomized block layout. Investigators often find themselves in the following situations: at time T, when it is necessary to end the experiment or at least evaluate the results up to that time, a certain number of the subjects in each block will still be alive. The data will then consist of actual lifetimes and censored observations representing lifetimes known only to exceed time T. The generalized Friedman test is useful in such studies. (2) Investigators often wish to compare a treatment and control with respect to a vector of responses. Overall differences in effects are often tested by using the multidimensional Wilcoxon-Mann-Whitney U-test. However, the investigators may also be interested in detecting which differences in component

effects are the major factors in determining significance of the overall test. The nonparametric confidence ellipsoid is useful in making such comparisons.

PROPOSED COURSE: To study the performance of the nonparametric confidence ellipsoid as compared to the Puri and Sen (1970) nonparametric confidence ellipsoid for Δ, the multidimensional location-shift parameter.

PUBLICATIONS

Patel, K. M. and Hoel, D. G.: A nonparametric test for interaction in factorial experiments. J. Amer. Statist. Assoc. 68, 615-620, 1973.

Patel, K. M. and Hoel, D. G.: A generalized Jonckheere k-sample test against ordered alternatives when obsersations are subject to arbitrary right consorship. Communications in Statist. 2, 373-380, 1973.

Patel, K. M.: Hájek-Sidůk approaches to the asymptotic sistribution of variate rank order statistics. J. Multivariate Analysis 3, 57-70, 1973.

Serial No.: NIEHS-B-008
1. Environmental Biometry Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Development of Pharmacokinetic Models"

PREVIOUS SERIAL NUMBER:

PRINCIPAL INVESTIGATORS: M. W. Anderson, Ph.D., T. E. Eling, Ph.D. (Pharmacology Branch), and H. B. Matthews, Ph.D. (Pharmacology Branch)

OTHER INVESTIGATORS: M. J. L. Carver, M.A.T. and T. A. Clemmer, M.A.

COOPERATING UNITS: Pharmacology Branch

MAN YEARS

Total:	2.1
Professional:	2.0
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: The basic objective is to understand the mechanisms which control the distribution, metabolism and excretion of chemicals in various species. The development of mathematical models helps to understand these mechanisms.

MAJOR FINDINGS: An isolated perfused lung system was developed so that initial rates of uptake and efflux of accumulated chemicals could be investigated. Using this system, detailed models for the dynamic interaction between the lung and several basic amines have been developed. For the exogenous amines, imipramine and methadone, a transport system is not involved in their uptake since the initial velocity of uptake is linearly related to concentration. Efflux studies show that several types of binding sites are responsible for the accumulation of these amines by the lung. These studies also showed that if the amine is allowed to accumulate, then some of it cannot be effluxed. This non-effluxable component of accumulated methadone or imipramine is being pursued in more detail. Although it has been previously demonstrated that 5-HT is detoxified by the lung, the system has permitted the first characterization of the dynamic aspects of the transport and the metabolic component of this system and of the interactions between them. The vasoactive hormone is removed from the circulation by a uni-transport system, metabolized by the intracellular monoamine oxidase to 5-hydroxy indole acetic acid and then the metabolite effluxes by diffusion back into the circulation. Mathematical models are being developed

which will allow the prediction of the dynamics of the accumulation of these amines by the lung for any given blood concentration. The methodology and techniques that have been developed can be used to study these aspects of lung interactions with any chemical.

The development of pharmacokinetic models for several PCBs is being attempted. In particular, feasibility of developing pharmacokinetic models is being investigated for these very lipophilic compounds similar to those constructed for several drugs by workers at NCI (Dedrick, Birchoff, Zarharbo, et al). These models are constructed from basic biochemical and physiological parameters. Analysis of time-distribution curves for the PCBs show that the liver plays a very important role in determining the distribution, metabolism and excretion into bile can be examined. This information will be used in developing the model. Other than the liver, the other important storage sites for the PCBs are muscle, fat and skin. The concentration ratio of the PCBs between muscle and blood remains essentially constant 10 minutes after an i.v. dose. Thus the mathematical equation for the muscle component is straightforward. At present, equations are being developed which will predict fat and skin levels if the blood concentrations are known as a function of time. The only parameters in these equations are the blood flow rates and a partition coefficient of the PCB between fat or skin and blood. After these various studies are completed, there will be available a pharmacokinetic model for the PCBs or an understanding why this type of model (Dedrick et al) cannot be developed for these compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Refer to NIEHS-P-010 and NIEHS-P-014, which are project reports of the Pharmacology Branch.

PROPOSED COURSE: Refer to NIEHS-P-010 and NIEHS-P-014, which are projects of the Pharmacology Branch.

PUBLICATIONS

Eling, T. E., Orton, T. C., Pickett, R. D., Anderson, M. W. and Fouts, J. R.: Xenobiotic accumulation in the isolated perfused rabbit lung. Federation Procs., Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 1973.

Orton, T. C., Anderson, M. W., Pickett, R. D., Eling, T. E.,and Fouts, J. R.: Xenobiotic accumulation and metabolism by isolated perfused rabbit lungs. J. Pharmacol. Exptl. Therap. 186: 482-497, 1973.

Pickett, R. D., Orton, T. C., Anderson, M. W. and Eling, T. E.: Accumulation of amines in the isolated, blood-perfused rabbit lung. The Pharmacologist 15: 2, 1973.

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ENVIRONMENTAL BIOPHYSICS BRANCH

ENVIRONMENTAL BIOPHYSICS BRANCH
Summary Statement

The Environmental Biophysics Branch was established and staffed as an intramural research branch during FY 74. The staff was transferred from the Biophysics Group in the Research Services Branch. The instrumentation development and maintenance component of this group was retained in the Research Services Branch. Staffing of the Environmental Biophysics Branch currently consists of an Acting Chief, eight scientists, four technicians and a secretarial vacancy.

Major research efforts of the Branch include studies on: (1) auditory and non-auditory effects of noise as well as the ototoxic effects of certain drugs in combination with noise; (2) development of non-ionizing radiation exposure systems, dosimetry techniques, and determination of non-thermal effects of non-ionizing radiation on biological systems; and (3) developing a theoretical basis for predicting dose-response relationships and exploring the use of X-ray emission spectrometry for quantifying elements in biological samples.

Noise

Dr. Teruzo Konishi joined the staff in December 1973 and is establishing an auditory physiology laboratory. The subject of auditory fatigue will be explored to delineate the physiological changes with a temporary or permanent threshold shift in hearing and to better understand the potentiation of these shifts with the introduction of certain ototoxic agents. Changes in cochlea energy utilization and production influenced by noise and ototoxic drugs will be studied utilizing electrophysiological and biochemical techniques. Dr. Konishi plans to collaborate with the microwave group in studying the effects and mechanisms of action of microwaves on the auditory system.

Drs. Fox, Falk and Cook have initiated studies to determine the influence of noise, continuous and intermittent, on responses of non-auditory systems. Hormonal secretions and phagocytosis function alterations will be looked for over time following noise stress.

Dr. Falk is concluding his work at the Institute and plans to begin a residency in surgery this summer at Colorado State University. Dr. Falk's three years at the Institute have been very fruitful and he will be missed. Drs. Falk and Cook have completed the initial study on comparing susceptibility of the cochlea of newborn and adult guinea pigs to high intensity noise. The morphological data disclosed significantly more damage to the cochlea hair cells of the young guinea pigs.

Dr. Cook is continuing to develop a piezoelectric or other direct contact (not bonded) transducer which will impart the same or equivalent signal to the cochlea as an acoustical wave when both are set to create equal maximum displacement of the ossicular chain.

The Kresge Hearing Institute contract is for determining the extent and nature

of the interaction between noise and ototoxic drugs in experimental animals, and to study the biochemical mechanisms involved. Work is progressing but no significant findings have been reported to date. The contract has another year plus to go.

Radiation

Dr. Walsh continued developing a mathmatical-theoretical model for describing the effects of ionizing radiation at the cellular level. During this past year a theoretical model was applied to experimental cell survival curves available in the literature. The parameters obtained from the survival data are the same parameters to be used for the description of cell alteration as a function of dose. If such a model is verified by further experimental work and by comparison with available data, it may be applicable to other physical, biological or chemical toxicants.

The development of respiratory tract dose models by contract with the Lovelace Foundation is progressing well. Morphometric measurements on lung casts of man and several animal species are complete. Physical models of respiratory tracts are being constructed. Aerosol particle deposition studies in animals have begun. Consideration is being given to amending this contract to include studies on deposition of SO_x in combination with particulates.

Microwaves

Dosimetry for microwaves in free fields and biological specimans which have been evaluated by Branch personnel and found to have utility for laboratory use include: (1) thermistors, (2) infrared microscope, and (3) liquid crystals. Other dosimetry techniques which will be further evaluated include: (1) pyroelectric and magnetic probes which may be implantible in tissues, (2) a pressure transducer-capillary dosimeter, and (3) fiber optic-liquid crystal probes.

Exposures of several biological systems to 2450 MHz CW microwaves have been completed. The power densities of these microwave exposures have been limited to that level required to maintain incubating temperature during exposure. In all cases, the exposure levels exceeded the accepted safe levels for humans by a factor of three and up to a factor of thirteen. The replication rates for bacteria and bacteriophage were not altered after exposure to microwaves over a 16 generation time frame. Japanese quail eggs were exposed for four hour periods each day during the first five days of embryonic development and held until hatching. No effects on hatching rates or gross deformaties were observed between exposed and controls. Small but statistically significant changes were observed in the hematocrit, hemoglobin and lymphocyte counts of the quail. DNA was exposed for a 16 hour period and no denaturation due to microwaves was observed. In all of these studies precautions were taken to prevent thermal and other external stressors from influencing the results. Modifications to the exposure systems are being undertaken to permit higher exposure levels while at the same time excluding thermal effects.

Studies are being initiated to start to look at effects of modulated and CW microwave radiation on the functioning of the central nervous system.

Serial No.: NIEHS-EB-001
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Acute Methyl Mercury Intoxication and Ototoxicity in Guinea Pigs"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: Ronald Klein, M.D., Joseph Haseman, Ph.D. and Fred Talley

COOPERATING UNITS: Environmental Toxicology Branch, Environmental Biometry Branch, Environmental Biology and Chemistry Branch

MAN YEARS

Total:	.5
Professional:	.5
Other:	.0

PROJECT DESCRIPTION

OBJECTIVES: To identify the pathological effects of methyl mercury hydroxide in the guinea pig.

METHODS EMPLOYED: Young Hartley guinea pigs were treated with daily subcutaneous doses of 2 mg Hg in the form of methyl mercury hydroxide per kg body weight five times per week for 1, 2, 4 and 6 weeks. Clinical examination, complete autopsy, and brain and blood Hg levels were performed.

MAJOR FINDINGS: The ototoxicity of methyl mercury has been reported separately in report NIEHS-RSB-013. The dose schedule produced a mild degree of intoxication. Clinically, only weight loss and decreased activity developed. Histologic examination revealed calcific foci in the cerebral cortex, pyknotic neurons of the internal granular layer of the cerebellum, mild degree of fatty metamorphosis in central lobular hepatocytes, and swelling of epithelial cells of proximal tubules of the kidney.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies indicate that the guinea pig is less susceptible than the rat to intoxication by methyl mercury. This is probably because of species differences in distribution and metabolism of methyl mercury. After 1 and 2 weeks treatment, guinea pigs have total mercury concentration in blood of 8.2 and 23.2

μg/ml, respectively. Under identical treatment regimen, concentrations of 49.1 and 78.0 μg/ml have been reported in rats after 1 and 2 weeks treatment, respectively. This represents a three to six fold greater concentration in rats compared with guinea pigs.

PROPOSED COURSE: This concludes our work in methyl mercury intoxication.

PUBLICATIONS

Falk, S.A., Haseman, J.K., Klein, R., Falk, M.K.: Methyl mercury ototoxicity a model for the surface preparation technique. The Laryngoscope 83:1769-1782, 1973.

Falk, S.A., Klein, R., Haseman, J.K., Sanders, G., Talley, F.A., and Lim, D.J.: Acute methyl mercury intoxication and ototoxicity in guinea pigs. Archives of Pathology, in press.

Serial No.: NIEHS-EB-002
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Investigation of Hearing Loss Threshold Curve for Young vs Mature Animals"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-012

PRINCIPAL INVESTIGATORS: Stephen A. Falk, M.D. and Reginald O. Cook

OTHER INVESTIGATORS: None

COOPERATING UNIT: None

MAN YEARS

Total:	.5
Professional:	.25
Other:	.25

PROJECT DESCRIPTION

OBJECTIVES: To compare susceptibility of the cochlea of newborn and adult guinea pigs to high intensity noise.

METHODS EMPLOYED: Two day (group A), eight day (group B), and eight month (group C) old guinea pigs were exposed to 30 continuous hours of white noise at 119-120 dB SPL. One month later pathology of the organ of corti was evaluated and quantitated by use of the surface preparation technique. Percent cell damage was determined for outer hair, inner hair, outer pillar, and inner pillar cells at each of the 4 turns of the cochlea and for the cochlea as a whole. Comparisons of pathology of each cell type were made between groups.

MAJOR FINDINGS: Mean percent outer hair cell damage per cochlea (± 1 S.E.) was 23.72 ± 3.69 for group A, 36.98 ± 5.76 for group B, and 7.24 ± 1.75 for group C. There was no significant difference in outer hair cell damage between groups A and B. Outer hair cells of groups A were significantly more damaged than those of group C when damage in the cochlea as a whole was considered due to significantly greater damage in group A at 3-1/2 turns. Likewise, outer hair cells of group B were significantly more damaged than those of group C when damage in the cochlea as a whole was considered due to significantly greater damage in group B at 2-1/2 and 3-1/2 turns.

A similar effect was observed in terms of pathology of inner hair cells and pillar cells: there was a trend toward increased damage in animals of groups A and B compared with C. Group C showed no outer or inner pillar cell damage, and only 1 of 6 animals had alterations in inner hair cells. In contrast, outer and inner pillar cells were damaged in groups A and B, and 4 of 6 animals of group A and 6 of 8 of group B showed inner hair cell damage.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
There is now morphological, electrophysiological, and audiometric evidence indicating greater susceptibility of young cochleas when compared with older cochleas to noise-induced physiological and pathological alterations. However, firm recommendations in terms of maximally allowable parameters of noise exposure for the prevention of noise-induced deafness in newborns cannot presently be made because of the paucity of information available on this problem. Nevertheless, at this time it would seem medically prudent to take extra precautions to avoid exposing newborns to excessive noise.

This report concludes this research project.

PUBLICATIONS

Falk, S.A., Cook, R.O., Haseman, J.K., Sanders, G.M.: Noise-induced Inner Ear Damage in Newborn and Adult Guinea Pigs. The Laryngoscope, in press.

Serial No.: NIEHS-EB-003
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Combined Effect of Noise and Salicylates on Cochlear Morphology and Auditory Threshold"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-023

PRINCIPAL INVESTIGATORS: Stephen A. Falk, M.D., Reginald O. Cook and Teruzo Konishi, M.D., Ph.D.

OTHER INVESTIGATORS: John R. Morgan, Ph.D. and Deborah Finn, M.S.

COOPERATING UNITS: Department of Physiology & Biophysics, Colorado State University and Department of Speech & Hearing Sciences, University of North Carolina

MAN YEARS:

Total:	1.25
Professional:	1.0
Other:	0.25

PROJECT DESCRIPTION

OBJECTIVES: To elucidate interactions between noise and salicylates in the production of hearing loss.

METHODS EMPLOYED: Twenty five young Hartley guinea pigs (average weight 300 grams) were divided into four groups. One group was exposed to 115 dB SPL (linear) white noise continuously for 96 hours, the second group received sodium salicylate (100 mg/kg body weight given as a single injection daily for six days), the third group was exposed to both the noise and sodium salicylate, and the fourth group served as the controls. Pathology in the organ of Corti was evaluated by use of the surface preparation technique and was compared to that of the control animals.

Chinchillas are used to study the growth and recovery of temporary threshold shift (TTS) from noise alone and from noise and salicylates by behavorial audiometry. Each animal is used as his own control and exposed to 80 dB (linear), 500 Hz octave band noise for 3 days continuously. The growth and complete recovery of TTS is followed. Then sodium salicylate 200 mg/kg body

weight is injected subcutaneously until the TTS stabilizes at 20-30 dB. Serum salicylates are then measured. Identical noise parameters as before are used and growth and recovery of TTS are studied.

This study will be a repeat of the study detailed in the first paragraph with the important addition of monitoring serum salicylate levels and cochlear electrical potentials. A wide variability of data was noted between animals in the group exposed to both noise and salicylates, even though the average was not significantly different from the control group. This variability may be attributable to different salicylate serum levels. Monitoring cochlear electrical potentials will give us another sensitive measure of cochlear function for correlation purposes.

MAJOR FINDINGS: In guinea pigs, overall outer hair cell damage was 1.82 ± .69 (SE)% in the controls, 2.96 ± 1.38 in the salicylate group, 18.30 ± 2.20 in the noise group, and 23.10 ± 3.96 in the salicylate plus noise group. Damage was primarily apical. The effect of sodium salicylate, either alone or in combination with noise, was not significant where assessed by morphological criteria. However, these studies are to be repeated with concomitant serum salicylate levels to assure that at least 20 mg% serum salicylate is achieved in the animals.

In the chinchilla study preliminary data indicate that the combined TTS from noise and salicylates is greater and has longer recovery until normal thresholds are reached than compared with the noise-induced or the salicylate-induced TTS separately. Pathological evaluation of the cochleas by the surface preparation technique will be shortly undertaken.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of any interaction between salicylates such as aspirin and noise is obviously of public health importance considering the enormous amounts of aspirin consumed. Our studies thus far indicate that a profound ototoxic interaction is not taking place, but a clinically significant interation is a real possibility on the basis of our preliminary data.

PROPOSED COURSE: Pathological evaluation of the chinchilla cochleas and repeat exposures will be done.

Serial No.: NIEHS-EB-004
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Noise in the Hospital"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-022

PRINCIPAL INVESTIGATOR: Stephen A. Falk, M.D.

OTHER INVESTIGATORS: Nancy F. Woods, RN, MN

COOPERATING UNITS: Dept. of Nursing
Duke University Medical Center
Durham, N. C.

MAN YEARS

Total:	.2
Professional:	.2
Other:	.0

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are to identify the sources and quantify the levels of excessive noise present in the hospital and to relate these levels to pathophysiological effects on the hospitalized patient.

METHODS EMPLOYED: A sound level meter with octave band analyzer was used to measure sound pressure levels in functioning infant incubators and in a 17 bed surgical recovery room and a 7 bed acute care unit (for post-operative care of cardiac surgical patients) consisting of room 1 and 2 at Duke Hospital. The predominant sources of the noise-equipment, personnel, procedures, etc.--were noted. To provide a complete evaluation of noise levels over 24 hours, measurements were made for 10 seconds at 5 minute intervals and were correlated with the number of hospital staff and patients and with other sources.

MAJOR FINDINGS: The average A-weighted and unweighted sound pressure levels of the incubators are 57.7 dB and 74.5 dB respectively with a frequency distribution between 31.5 and 250 Hz. The levels of noise generated by individual mechanical equipment, personnel, and patients averaged 65.6 dB(A) and 80.0 dB (linear) and emanated from multiple sources including suction machines, respirators, cardiac monitors, oxygen outlets, bedpan washer, moving beds, X-raying patient, deliverying supplies, child crying, and suctioning patients. Some noise sources were necessary, others were not. The 24 hour noise surveys revealed that the average noise level in the recovery room is higher during

the day than night with an average of 59 dB(A) for 7:00 a.m. to 3:00 p.m., 58 dB(A) for 3:00 p.m. to 11:00 p.m. and 55 dB(A) for 11:00 p.m. to 7:00 a.m. The range is 45-84 dB(A). The range in room 1 and 2 of the acute care unit is 50-70 dB(A) and 50-76 dB(A).

In all locations, noise level is highly correlated with number of staff, and in the recovery room, noise is also associated with number of patients. If attention is restricted to periods having at least one patient, the average noise levels are elevated to 60.1 dB(A) and 73.3 dB (linear) for room 1 and 55.8 dB(A) and 68.1 dB (linear) for room 2.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These noise levels were evaluated for adverse effects on the hospitalized patient by careful consideration of our present knowledge of the physiological effects of noise on the pituitary-adrenal axis and the cardiovascular and auditory systems.

Like other stressful stimuli, such as trauma, surgery, infection, and burns, noise can be considered a nonspecific stress which releases ACTH from the pituitary. A level of noise as low as 68 dB (linear) (which is less than the average noise present in the recovery room, acute care unit, and incubators in this study) for only 30 minutes releases ACTH as measured by a decrease in adrenal ascorbic acid content and by eosinopenia, a peripheral glucocorticoid effect. In humans, a 65 dB sound at 10,000 Hz causes a 53% increase in plasma 17-hydroxy-corticosteroids. There is indirect evidence that noise-induced adrenal changes are transient, disappearing with cessation of the noise. However, there is no evidence that the hypothalamo-hypophyseal-adrenal axis adapts to the stress of chronically maintained noise.

Although data in humans is incomplete, it is highly probable that noise levels as measured in this study stimulate the hypophyseal-adrenocortical axis of hospitalized patients. Because these patients are already in stressful situations from multiple causes including surgery, infection, hemmorrhage, and fear, it would appear wise to decrease any further stress to which they are exposed.

Our evaluations of the adverse cardiovascular effects of hospital noise indicate that hospital noise exceeds the threshold of 70 dB (linear) for peripheral vasoconstriction.

Based on present criteria of 80 dB(A) for the production of hearing loss, the hospital noise levels measured in this study are not a danger to hearing. However, these criteria are not applicable to the hospitalized patient because they are based on intermittent 8 hour per day exposures, not continuous ones, which are present in the hospital and which are more damaging than intermittent which provides an opportunity for recovery. Also they are based on studies in adults and do not strictly apply to infants. Also recent studies demonstrating potentiation of the damaging effects of noise and ototoxic aminoglycosidic antibiotics can be applied to hospitalized patients. A much larger amount of hair cell damage in the organ of Corti occurs in guinea pigs upon exposure to kanamycin and noise levels of 58 dB(A) compared to exposure to either agent alone. The amount of damage greatly exceeds that expected from adding the separate effects of each agent. A similar level of noise is also present in the incubators, recovery room and acute care unit in this study.

Recommendations for decreasing noise levels were arrived at. A lowering of noise levels can be achieved by improved acoustical design of existing facilities and better acoustical consultation in erecting new facilities. Also physicians and nursing personnel can lessen unnecessary noise levels.

This report concludes this research and project number.

PUBLICATIONS

Falk, S. A. and Woods, N. F.: Hospital noise: levels and potential health hazards. N. Engl. J. Med. 289:774-781, 1973.

Falk, S. A. and Woods, N. F.: Hospital noise. N. Engl. J. Med. 290:522, 1974.

Serial No.: NIEHS-EB-005
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Simultaneous Measurement of Ossicular Chain Movement, Free Field Sound Pressure Level at the Tympanic Membrane and Cochlear Electrical Potentials in Response to 1) Sound and 2) Piezoelectric and other Direct Contact Tranducers"

PREVIOUS SERIAL NUMBER: This project results from the combination of projects NIEHS-RSB-024 and NIEHS-RSB-009

PRINCIPAL INVESTIGATOR: Reginald O. Cook

OTHER INVESTIGATOR: Teruzo Konishi, M.D., Ph.D.

COOPERATING UNIT: None

MAN YEARS

Total:	.43
Professional:	.33
Other:	.10

PROJECT DESCRIPTION

OBJECTIVE: Overall objective is to ascertain whether a piezoelectric or other direct contact (but not bonded) transducer imparts the same signal to the cochlea as an acoustic wave when both are set to create equal maximum displacement of the ossicular chain.

METHODS EMPLOYED: Using techniques heretofore developed in projects NIEHS-RSB-009 and NIEHS-RSB-024 along with differential electrode cochlear microphonic measurements we can determine whether the eardrum-ossicular chain leaves the tranducer on the "return" phase. Displacements will be measured with non-contact electro-optic techniques and sound with probe tube microphones. Animals used will be guinea pigs and/or cats.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We have demonstrated capability to meausre small deflections 3-5 A across frequency without use of an expansive laser interferometer and have developed techniques for building piezoelectric transducers capable of delivering complex audio frequency signals at the angstrom displacement levels needed by the ear. It remains to be proven whether the mechanical characteristics (damping, etc.) of the ear allow the ossicular chain to precisely follow the

complex wave forms necessary for distortion free speech recognition from direct contact but non-bonded transducers. This experiment will give us insight into whether such transducers, which can more precisely mimic electrical wave forms than acoustical transducers, are acceptable or superior for auditory input-output research.

A paper on earlier phases of this work was given at the annual meeting of the Acoustical Society of America.

Serial No.: NIEHS-EB-006
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Development of a Double Beam Laser Microscope for Recording Ossicular Chain and Basilar Membrane Displacement and Waveform"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Reginald O. Cook

OTHER INVESTIGATORS: Dr. Geza Jako and Teruzo Konishi, M.D., Ph.D.

COOPERATING UNIT: Boston University School of Medicine

MAN YEARS

Total:	.55
Professional:	.25
Other:	.30

PROJECT DESCRIPTION

OBJECTIVES: Objective is to develop a calibrated electro-optic microscope utilizing twin laser beams so that phase and amplitude differences for two closely adjacent points on the vibrating basilar membrane can be simultaneously measured. Amplitude sensitivity sought is on the order of 1 angstrom unit.

METHODS EMPLOYED: Using a metallurgical microscope and a high power, long working distance lens coupled with twin laser beams brought "together" by use of a rhombsidal prism mechanism and a state of the art electro-optic photo sensor, we have already achieved ability to measure displacements of 1 Å off the less reflective basilar membrane. A paper detailing the method will be given at the May meeting of the Acoustical Society of America.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Using this technique, we may be able to quantitatively characterize basilar membrane response to sound input. Such quantitative characterization has been a primary goal of auditory physiology workers for many years since only through such measurements can theories involving the complex mechano-electro-neural relationships in hearing and its deterioration be put on a firm foundation.

Serial No.: NIEHS-EB-007
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Ototoxcity of Methyl Mercury"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Teruzo Konishi, M.D., Ph.D.

OTHER INVESTIGATOR: Reginald O. Cook

COOPERATING UNIT: None

MAN YEARS

Total:	.24
Professional:	.14
Other:	.10

PROJECT DESCRIPTION

OBJECTIVES: To study the effect of methyl mercury on cochlear potentials in guinea pigs and to correlate the functional alterations with morphological changes in the cochlea.

METHODS EMPLOYED: Guinea pigs are treated with daily subcutaneous doses of 2 mg/kg of body weight for a period of 4 to 6 weeks. Cochlear potentials and electrolyte contents (Na and K) in the endolymph are measured. The cochlear damage is assessed by cochleographic reconstruction.

MAJOR FINDINGS : The auditory physiology laboratory has been recently developed and there are no major findings to report at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In previous studies (Falk, et al 1973) it was found that methyl mercury has a selective toxicity to the outer hair cells in the middle turn of the guinea pig cochlea. This project will provide additional knowledge regarding the ototoxic action of methyl mercury and the etiology of sensorineural deafness induced by methyl mercury.

PROPOSED COURSE: This project will be carried out as outlined.

Serial No.: NIEHS-EB-008
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Physiological Study of Auditory Fatigue

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Teruzo Konishi, M.D., Ph.D.

OTHER INVESTIGATOR: Reginald O. Cook

COOPERATING UNIT: None

MAN YEARS

Total:	.64
Professional:	.34
Others:	.30

PROJECT DESCRIPTION

OBJECTIVES: The principal goal is 1) to explore the loci or nature of the physiological changes underlying the temporary or permanent threshold shift and 2) to obtain physiological knowledge regarding potentiation of auditory fatigue by subtoxic doses of antibiotics.

METHODS EMPLOYED: The cochlear microphonic, whole-nerve action potential and nerve impulses of single auditory-nerve fiber, are recorded and measured in anesthetized guinea pigs or cats. The recovery processes of the receptor potential and neural responses are examined after sound exposure. The effects of both exposure tones (duration, intensity and frequency) and test tones (intensity and frequency) on the recovery curve are systemically tested. The same experimental paradigms are used in guinea pigs or cats which are treated with subtoxic doses of kanamycin (200 mg/Kg, 5 days) in order to test the possible potentiation effect of the antibiotic on the auditory recovery process after sound exposure.

MAJOR FINDINGS: The auditory physiology laboratory has been recently developed and there are no significant findings to report at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ability of an ambient sound to produce permanent hearing loss over a long period of time can be predicted from the temporary threshold shift. Many properties of temporary threshold shift have been reported in psychoacoustic studies, but these studies provide little information about the nature of

the physiological changes underlying the temporary threshold shift. The knowledge obtained by this project is important for an understanding of the physiological process upon which the temporary threshold shift depends.

PROPOSED COURSE: This project will be carried out as outlined, since it has been only recently proposed.

Serial No.: NIEHS-EB-009
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Studies of Energy Balance and Metabolism in Cochlea Affected by Noise, Ethacrynic Acid and Other Ototoxic Agents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Teruzo Konishi, M.D., Ph.D.

OTHER INVESTIGATORS: Insu P. Lee, Ph.D. and Reginald O. Cook

COOPERATING UNIT: None

MAN YEARS

Total:	.73
Professional:	.43
Other:	.30

PROJECT DESCRIPTION

OBJECTIVES: 1) To study changes in energy utilization and production under the influence of known ototoxic agents (e.g. noise, ethacrynic acid, etc.) by utilizing electrophysiological, bio-chemical and radioactive tracer techniques and 2) to pinpoint the specific mechanism by which these actions occur in the cochlea.

METHODS EMPLOYED: The cochlear microphonic and whole-nerve action potential are recorded in anesthetized guinea pigs. The endocochlear potential is monitored in the scala media. Samples of the endolymph and perilymph are collected after exposure to ototoxic agents. The permeability of the cochlear partition to the radioactive Na, K and Ca is determined. ATP levels are assayed by microtechnique.

MAJOR FINDINGS: The aduitory physiology laboratory has been recently developed and there are no major findings to report at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Ototoxcity of the subject agents has been studied but the bio-chemical basis underlying the suppression of the cochlear potentials is not fully understood, nor have attempts been made to simultaneously measure functional (electrophysiological) and underlying bio-chemical changes. The results obtained by this project may permit us to identify and begin to characterize the basic mechanism associated with cochlear pathology and resulting hearing loss.

PROPOSED COURSE: This project has been recently proposed and will be carried out as outlined.

Serial No.: NIEHS-EB-010
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effect of Microwave Radiation on Auditory System"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Teruzo Konishi, M.D., Ph.D.

OTHER INVESTIGATORS: Donald I. McRee, Ph.D.
Philip E. Hamrick, Ph.D.
Reginald O. Cook

COOPERATING UNIT: None

MAN YEARS

Total:	.32
Professional:	.17
Other:	.15

PROJECT DESCRIPTION

OBJECTIVES: 1) To determine the effects of incident microwaves on the auditory system of experimental animals and 2) to investigate the physical mechanism of microwaves in order to elucidate the auditory excitation.

METHODS EMPLOYED: The cochlear microphonics and whole-nerve action potentials are recorded in the guinea pig cochlea. The intracochlear and intracranial temperatures are measured by thermisters. Animals are exposed to modulated microwaves of frequencies from 1 to 10 GHz. The stimulus-response relationships are tested. Electrical responses to microwaves are examined after labyrinthectomy in order to explore the possible mode of microwave-induced auditory responses.

MAJOR FINDINGS: The auditory physiology laboratory has been recently set up and there are no major findings to report here.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Microwave radiation has been viewed as a source of thermal effects on biological structures. Its effect on the auditory system has not been explored extensively. It is therefore of importance to study these effects on the auditory system and to investigate the nature of functional alteration of the auditory system induced by microwave radiation.

PROPOSED COURSE: This project will be carried out as outlined.

Serial No.: NIEHS-EB-011
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Effects of Noise on Corticosterone Secretion in the Rat"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Stephen Falk and Sherman Fox

OTHER INVESTIGATOR: Reginald O. Cook

COOPERATING UNIT: None

MAN YEARS

Total:	0.4
Professional:	0.3
Other:	0.1

OBJECTIVES: To study and compare the effects of chronic noise, both continuous and intermittant, on rat adrenal corticoid secretion, as well as long term effects after cessation of noise.

METHODS EMPLOYED: One hundred twenty young male rats were weight matched and assigned to constant noise, intermittant noise, and control groups. The noise level was 100 dBA, and the intermittant noise consisted of two five minute periods per hour, for fifteen consecutive hours per day. Five animals from each group were sacrificied just before onset of exposure; after one hour, four hours, 24 hours, one week and two weeks of noise; and at one and four weeks after cessation of noise. At time of sacrifice, serum was taken and frozen for subsequent corticosterone radioimmune assay, and adrenal, thymus, liver, and peripheral lymph nodes were taken and fixed for histological study.

MAJOR FINDINGS: Assays are now in progress, thus there are no findings to be reported at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Among the many acute hormonal effects of noise is an increase in adrenal costicoid secretion. No knowledge now exists concerning corticoid response over period of noise exposure greater than 24 hours, nor after cessasion of noise. This study looks at parameters more closely related to actual environmental noise exposure, namely, chronicity and intermittancy, as well as at possible long term, post-exposure effects.

PROPOSED COURSE: Corticosterone samples on constant-noise-exposed animals are in the process of being assayed. Those on intermittant noise exposed animals are now being collected.

Serial No.: NIEHS-EB-012
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Noise and Polymorphonuclear Leukocyte Function"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: Sherman Fox and Joseph Zinkl

OTHER INVESTIGATORS: Reginald Cook

COOPERATING UNIT: None

MAN YEARS

Total:	0.5
Professional:	0.25
Other:	0.25

PROJECT DESCRIPTION

OBJECTIVES: Studies of immune response have classically focused their attention on lymphocyte function. Another important facet of the defense system is the PMN. Various environmental agents have been shown to affect PMN function. One study done with noise showed a detrimental influence on the exudative inflammatory process, which may have been due to PMN dysfunction. In this study we shall directly study the effects of noise of differing duration, both constant and intermittant, on chemotaxis, phagocytosis and bactericidal activity.

METHODS EMPLOYED: Chemotaxis will be determined by a modification of the Boyden technique using a miniature chemotaxis chamber. The chamber consists of upper and lower compartments separated by a milipore filter. PMNs from the experimental animals will be placed in the upper compartment and leukocyte chemotactic factor in the lower. After appropriate incubation, the member of cells that passed from one side of the filter to the other will be determined.

Tests of particle uptabe will be performed by mixing a suspension of leukocytes with inert particles. After an appropriate incubation, the average number of particles ingested per PMN will be determined.

Bactericidal activity will be measured by mixing a suspension of leucoytes with Staphlococci in the presence of fresh serum. After an appropriate length of time, the amount of residual bacteria remaining within cells will be measured by direct culture technique.

Male rats will be weight matched and placed into three groups constant noise, intermittant noise and controls. The constant noise group will be exposed to 100 dBA for 24 hours per day. The intermittant noise group will be exposed to two five minute periods of 100 dBA noise for fifteen consecutive hours per day. The control group will be exposed to ambient noise. At one hour, four hours, one day, one week and two weeks after onset of exposure, animals from each group will be taken and the above PMN functions will be assayed.

MAJOR FINDINGS: There are no findings to be reported at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This is an attempt to better understand the influences of environmental stresses on the host-parasite relationship.

PROPOSED COURSE: We are in the process of perfecting the experimental techniques for the various assays.

Serial No.: NIEHS-EB-013
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Noise and Cell Mediated Immunity"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Sherman Fox

OTHER INVESTIGATOR: Reginald O. Cook

COOPERATING UNIT: Dept. of Microbiology, University of North Carolina

MAN YEARS

Total:	1.3
Professional:	.9
Other:	.4

PROJECT DESCRIPTION

OBJECTIVES: In the experimental animal, acute noise exposure has been shown to markedly affect various hormonal secretions, among them adrenal steroids and thyroid hormones. Immunologic function has been shown to be affected by changes in levels of these hormones. This serves as a rationale for studying possible effects of noise on immune system function. Work in the early 1960's showed that prolonged high intensity noise increased the susceptibility of mice to viral infections. This was later shown to correlate with impairment of interferon production. Subsequent work by Russian scientists has shown various immunological dificiencies, both in experimental animals and in human workers, after chronic noise exposure. The objective of this project is to attempt to replicate the Russian findings concerning noise and cell-mediated immunity, and to further delineate the locus of action.

METHODS EMPLOYED: Cell-mediated immunity will be measured in male rats in vivo by de novo sensitization to PPD. Sensitization will be attempted during various patterns of constant and intermittant noise. The noise level will be 100 dBA, and the intermittant noise will consist of two, five minute periods per hour, 15 consecutive hours per day.

Cell mediated immunity will also be measured in vitro by lymphocyte stimulation by phytohemagglutinin. This will be measured by a micromethod, requiring only 0.5 ml of peripheral blood. As a result, we'll collect sequential data on a single animal during lengthly noise exposure. Again, both constant and intermittant noise will be looked at.

MAJOR FINDINGS: Pilot work with various patterns of noise exposure has indicated that sensitization does not require "noise-sensitive" precursors. Constant noise exposure between sensitization and challenging dose, however, has led to a statistically significant decrease in the amount of reaction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This is an attempt to better understand the influence of environmental stresses on the host-parasite relationship. In addition, it is an attempt to study one parameter of noise, intermittancy, that more closely approximates its environmental occurence than previous experimental models.

PROPOSED COURSE: We are now attempting to replicate our data, and shall then study intermittant noise. We are in the process of perfecting the technique for the lymphocute stimulation work.

Serial No.: NIEHS-EB-014
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Theoretical Basis of Dose-Response Relationships"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-002

PRINCIPAL INVESTIGATOR: P.J. Walsh, Ph.D.

OTHER INVESTIGATORS: P.E. Hamrick, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	2.0
Professional:	1.0
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to develop a mathematical-theoretical model of the effects of ionizing radiation at the cellular level.

METHODS EMPLOYED: Experimental and theoretical approaches are being developed. Experimental systems incorporating spectrometry systems have been designed to facilitate the delivery of measured doses of alpha, beta or gamma radiation to cell cultures. A theoretical treatment, built upon data from the literature, is being used to focus the experimental work and to speculate on the nature of dose-response relationships under low-dose, low-dose rate conditions.

MAJOR FINDINGS: During FY 74, a theoretical model was applied to experimental cell survival curves available in the literature. This model treats the relationships among such parameters as dose, dose rate, cell turnover time and altered cell division and repair. The parameter "n" from "multi-hit" or "multi-target" theory surfaces as a function of several variables. Also the parametric values obtained from cell survival data may be used to describe cell alteration as a function of dose. For example, the well-known "inverted-U" dose-response relationship for cancer arises naturally from the model.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Further information on the nature of interactions of ionizing radiation with matter is necessary for more accurate dose calculation and for improving our

understanding of the connections between damage to atoms and molecules and subsequent biological effects. Effort must be applied to the development of realistic models since it may be impossible to obtain sufficient experimental data especially under low dose conditions.

PROPOSED COURSE: In addition to, and as an aid to model development, the proposed course is to:

1. Study the energy loss characteristics of radiation in cell suspensions and cell cultures and develop quantitative dosimetric concepts with describe the results;

2. Deliver known doses of radiation to cells at various dose rates to determine dose-response relationships as a function of dose rate and turnover time of the cells;

3. Study the relative effectiveness of alpha, beta and gamma radiation for a given end point;

4. Use chemical or biological toxicants or protectors in conjunction with the radiation. Such studies should identify the most important molecular components in terms of biological response.

PUBLICATIONS

Walsh, P.J.: On the application of the uncertainty principle to stopping power theories. Health Physics 19:574, 1970.

Walsh, P.J.: Stopping power and range of alpha particles. Health Physics 19:312-316, 1970.

Walsh, P.J.: Possible effects of dose rate and turnover time of cells on dose response relationships: implications for radiation protecting guidance. International Journal of Environmental Studies 2:189-193, 1971.

Walsh, P.J.: Energy loss of alpha particles in tissue equivalent plastic. Health Physics 23:701-704, 1972.

Walsh, P.J.: Possible effects of division and repair of altered cells on dose response relationships in the context of carcinogenesis. International Journal of Environmental Studies. (in press)

Serial No.: NIEHS-EB-015
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Multielement Analysis Using X-ray Emission Spectrometry"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-017

PRINCIPAL INVESTIGATORS: P. J. Walsh, Ph.D. and P. Hamrick, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	.8
Other:	.2

PROJECT DESCRIPTION

OBJECTIVES: This project was initiated to develop quantitative techniques for the determination of trace amounts of elements, singly and in combination, in biological and environmental samples.

METHODS EMPLOYED: X-ray emission spectrometry has the advantage of speed of analysis because of its ability to analyze many elements simultaneously and its high detection efficiency. In recent years, the combination of semiconductor detectors with field-effect-transistor input preamplifiers and the optimization of that combination has yielded the resolution necessary to allow separation of the characteristic X-rays for all elements above atomic number 12.

MAJOR FINDINGS: A system incorporating on lithium-drifted germanium detector provides a minimum detectability of 2 μgm in 100 sounds. A new geometry and an intrinsic germunium detector provide greater flexiblity and promise better sensitivity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aside from its value as a conventional analytical tool, X-ray emission spectrometry offers the possibility of *in vivo* studies in animals and man ranging from the study of the distribution of iodine in thyroid glands to measurement of deposition of aerosols in the respiratory tract. A variety of studies normally carried out by the use of radioactive tracers appear within the capabilities of X-ray emission spectrometry.

PROPOSED COURSE: We plan to continue the development of X-ray emission spectrometry for use in NIEHS programs as the need for this method grows.

PUBLICATIONS

Walsh, P. and Hamrick, P.: Application of x-ray emission spectrometry to the determination of mercury in biological samples. Review of Scientific Instruments 44: No. 8, 1019-1020, 1973.

Serial No.: NIEHS-EB-016
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Measurement of Energy Absorption from Microwave Fields"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-021

PRINCIPAL INVESTIGATOR: P. J. Walsh, Ph.D.

OTHER INVESTIGATORS: Daryl Mercer, Graduate Student, UNC
Ravi Matthew, Graduate Student, UNC

COOPERATING UNITS: Department of Environmental Sciences and Engineering
University of North Carolina

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Measurement of the absorption of microwave energy on the basis of fundamental physical laws.

METHODS EMPLOYED: Microwaves are absorbed in a spherical shell of dielectric material enclosing a dry gas or a spherical shell of dry gas enclosing the dielectric. Temperature changes of the dielectric are transmitted to the gas which expands in accord with the ideal gas law over the range of pressures and temperatures encountered.

MAJOR FINDINGS: A double-walled spherical glass bulb in current use with saline dielectric and dry air gas exhibits sensitivities of 100-200 μ W/cm^2, a factor of 100 below current U. S. exposure limits.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTES: Measurement of dose or absorbed energy is generally more desirable than measurement of field intensity. A simple device which allows measurement of absorbed energy as well as exhibits adherence to basic physical laws has been a primary goal in radiation dosimetry.

PROPOSED COURSE: The utility of the system for determining the microwave absorption properties of various materials will be investigated.

Serial No.: NIEHS-EB-017
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Microwave Exposure System and Microwave Dosimetry"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-005

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATORS: P. J. Walsh, Ph.D.

COOPERATING UNIT: None

MAN YEARS

Total:	0.80
Professional:	0.50
Other:	0.30

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to develop a microwave exposure system for biological material which has a well-defined, uniform field characteristic; and to develop dosimetric relationships for energy absorption.

METHODS EMPLOYED: A thorough study of the research requirements on the biological effects of microwaves was completed. Based on these studies, a set of specifications was written for a microwave exposure system which would generate a uniform, low-level microwave field in a small laboratory space. At present, it is difficult to accurately measure low level microwave fields and to determine the mechanisms by which the fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, chemical reactions, liquid crystals and photographic film are some of the technics which will be investigated.

MAJOR FINDINGS: A microwave exposure system with a frequency of 2450 MHz has been designed, built and the field calibrated. The microwave field at the specimen location was determined to be uniform within the design specifications with variation in power density as follows:

6 inch diameter area - ±5%
9 inch diameter area - ±10%
12 inch diameter area - ±15%

The system provides a power density range of 0.01 mW/cm^2 to 200 mW/cm^2.

Thermistors and thermocouples have been evaluated for interaction with the microwave field. The thermistor and thermocouples were placed in the field with only air as the surrounding medium. The thermistor was not affected whether the leads to the thermistor bead were or were not shielded and/or grounded. The thermocouple was very sensitive to microwave interference if the leads were not grounded. Even when grounded some instability was noted. In future absorption and biological effects research, the thermistor will be used to monitor temperature.

An infrared microscope which can focus to a spot size of 0.25 cm at 52 cm from the aperture was obtained and evaluated. The surface temperature of objects being exposed to microwaves can be measured to ±0.5°C using this instrument. Liquid crystals with a range of 33-37°C were tested for their utility and accuracy. In this temperature range it was found that the surface temperature could be obtained to an accuracy of ±1°C.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The well defined microwave field will allow the Institute to perform accurate quantitative studies of the effects of microwave radiation in biological systems at a frequency of 2450 MHz. Using the thermistor to measure the temperature in the controls using ordinary heating techniques. This procedure will enable us to separate thermal and non-thermal effects.

PROPOSED COURSE: The pressure transducer-capillary detector which has been fabricated will be tested as to its accuracy, sensitivity, and response time. Implantible electric and magnetic field probes will be investigated as they become available. A prototype fiber optic-liquid crystal probe developed by the University of Utah and Office of Naval Research will be evaluated in our laboratory. It is reported that this probe provides an accurate measure of temperature and does not perturb the field at the measurement location.

PUBLICATIONS

McRee, D. I.: Thresholds for lenticular damage in the rabbit eye due to single exposure to CW microwave radiation: an analysis of the experimental information at a frequency of 2.45 GHz. Health Physics Journal, Vol. 21, Dec. 1971.

McRee, D. I. and Walsh, P. J.: Microwave exposure system for biological specimens. The Review of Scientific Instruments, Vol. 42, Number 12, Dec. 1971.

McRee, D. I. and Pendergrass, F. T.: Interaction of a 2450 MHz microwave field with thermocouples and thermistors. Health Physics, Vol. 25, August, 1973.

McRee, D. I.: Determination of the absorption of microwave radiation by a biological specimen in a 2450 MHz microwave field. Health Physics. (In Press)

Serial No.: NIEHS-EB-018
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Variable Frequency Microwave Exposure System"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-008

PRINCIPAL INVESTIGATOR: D. I. McRee, Ph.D.

OTHER INVESTIGATOR: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.40
Professional:	0.20
Other:	0.20

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to design, build and calibrate a variable frequency exposure system for biological material.

METHODS EMPLOYED: Specifications were written for the desired system. A field uniformity at the specimen of 90 percent over a 4-inch diameter circle, a continuous frequency variation in the range of 1 to 10 GHz and a power level range at the specimen of 0.010 to 10 mW/cm^2 were specified.

MAJOR FINDINGS: Many of the biological effects of microwave radiation are frequency dependent. In terms of whole body irradiation, a frequency of 150-1,200 MHz affects the internal organs, whereas, a frequency of 10,000 MHz and above affects primarily the skin. Components of cells such as RNA, DNA and protein were found to have maximum absorption at different frequencies. Therefore, it is necessary to have the capability of varying frequency when investigating microwave effects on biological systems. The frequency range of 1 to 10 GHz was chosen because it is within this range of frequencies to which the general population is exposed.

All components for the system have been obtained and assembled. It was necessary to order 4 three-port circulators and a triple stub turner to protect the TWT-amplifiers and obtain maximum power output. These components were not in the original design, but have now been obtained and incorporated into the system. A broad-band field probe was obtained from the National Bureau of Standards and the system calibrated between 1 and 6 GHz.

With this probe, the field patterns at the specimen were 90% uniform over a 4-inch diameter circle. Power levels of 20 mW/cm^2 were obtained through this frequency range. The pin modulators are now available which provide the added capability of modulating the microwave field.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many biological effects of microwave radiation appear to be frequency specific. The variable frequency exposure system will provide the researchers of the Institute the capability of studying the effects of different frequencies and modulated signals on biological systems.

PROPOSED COURSE: A microwave field probe will be obtained which will allow the calibration of the system from 6 to 10 GHz. The exposure area of the system will be placed in an absorber-lined environmental chamber. This will allow the exposure to be varied while maintaining a specified temperature in the specimen and humidity surrounding the specimen.

Serial No.: NIEHS-EB-019
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effects of 2450 MHz CW Microwave Radiation on the Embryonic Development of Japanese Quail"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-020

PRINCIPAL INVESTIGATORS: P.E. Hamrick and D.I. McRee

OTHER INVESTIGATORS: J. Zinkl, EBCB, P. Thaxton, C. Parkhurst, NCSU

COOPERATING UNITS: EBCB and Poultry Science Department of NCSU(consultants)

MAN YEARS

Total:	1.2
Professional:	0.8
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project is to determine the effects of 2450 MHz CW microwave radiation on the embryological development of Japanese quail.

METHODS EMPLOYED: Fertilized Japanese quail eggs were exposed to 2450 MHz microwave radiation for four hours per day during the first five days of incubation. A power level (30 mW/cm^2) was chosen that would hold the temperature of the eggs at incubation temperature (35-37^oC). The newly hatched quail were examined for changes in percent hatched, gross deformaties and several blood parameters. The blood parameters studied were hermatric hemoglobin, erthyrocyte number and differential leucocyte percentages.

MAJOR FINDINGS: Reported data and literature on the exposure of fertilized chicken eggs to microwave radiation has shown gross deformities. Due to the power density levels and the temperature of the eggs during exposure, there is controversy over whether the effects were thermal or nonthermal. In our experiments, to date, we have not allowed the temperature of any part of the exposed egg to be greater than 37^oC. Monitoring was done with thermistors and with a infrared microscope. Eggs had to be chosen near the same size to obtain essentially uniform heating. Even with careful selection of the eggs, some of the eggs had a temperature of 3 to 4^oC below that of a high at 37^oC. No effect on hatching or gross deformities was observed. Significantly lower (0.05) values for hemoglobin hermatric

and lymphocytes was seen for exposure on the second and third day of incubation. Although the average values were significantly lower, there is a large variability in the blood parameters of young quail and the observed lower values are still well in the range of normal variation. We therefore conclude that for the conditions for exposure given at 2450 MHz microwave radiation does preclude the essentially normal development of Japanese quail.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evaluation of whether microwave radiation affects differentiating cell systems is a basic question which must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

PROPOSED COURSE: Exposure of quail at 2450 MHz microwave radiation will be continued using exposure times and power levels.

PUBLICATIONS

D.I. McRee, P. Hamrick, J. Zinkl, P. Thaxton and C. Parkhurst, Some Effects of the Japanese Quail Embryo to 2.45 GHz Microwave Radiaition, presented at the New York Academy of Sciences Meeting in New York, February, 1974 and to be published in the annals of the Society.

Serial No.: NIEHS-EB-020
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: Effects of 2450 MHz Microwave Radiation on Biological Material at Cellular and Macromolecular Level

PREVIOUS SERIAL NUMBER: NIEHS-RSB-016

PRINCIPAL INVESTIGATOR: P.E. Hamrick, Ph.D.

OTHER INVESTIGATORS: J. Zinkl, EBCB, R. Staples, ETB

COOPERATING UNITS: EBCB and ETB

MAN YEARS:

Total:	0.8
Professional:	0.3
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and a macromolecular level, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects.

METHODS EMPLOYED: Several strains of bacteria, one bacteriophage and solutions of DNA have been exposed to 2450 MHz microwaves at power levels sufficient to maintain a temperature of 37°C. Bacterial growth rate curves and bacteriophage lysis times have been compared in exposed and control situations. Thermal denaturation curves of control and exposed DNA solutions have been compared with the aid of an ultraviolet spectrophotometer equipped with a temperature programmer. Embryos from mice were exposed for 22 hours in test tubes at 100 mW/cm^2 during the 2 to 4 cell stage and implanted in other mice for continued development. Washed rabbit erythrocytes have been exposed at several power levels and the cells examined for leakage of potassium and hemoglobin and changes in osmotic resistance.

MAJOR FINDINGS: Studies have been made which suggest that "non-thermal" microwave fields (fields which do not significantly increase the temperature of the specimen) at various frequencies interact with intracellular components to alter metabolic and/or genetic processes. Interpretation

of these effects is difficult because mechanisms are not specified and in many cases the exposure parameters are not known quantitatively. Many of the effects appear to be very frequency dependent. For example, the growth rate of Escherichia coli has been shown by others to be enhanced or retarded depending on the frequency in the range 65-75 GHz. When Escherichia coli and Pseudomonas aeruginosa were exposed in our lab to 2450 MHz microwaves at 60 mW/cm^2 and held at 37^{o}C, no effects on growth rate were observed. Under similar conditions no effect was observed on the lysis time of bacteriophage T-2 or on the thermal denaturation curves of DNA. Thus far, our studies indicate that if there are "non-thermal" effects at 2450 MHz then these effects are relatively small in the systems we have investigated. Growth rate, phage production and lysis time are systems very sensitive to disturbing influences because the interaction of many cellular components is involved. The sensitivity of the DNA solutions to thermal effects was varied by adjusting the ionic strength of the solutions. However, no sensitivity to microwaves has been detected at 2450 MHz and with exposure levels up to 200 mW/cm^2. Control and exposed mouse embryos were held at 37^{o}C during the 22 hour exposure period. After exposure, control embryos were implanted in one uterine horn and exposed in the other. The embryos were examined near the end of the gestation period for resorption and malformation. No differences were observed between exposed and control embryos. Polish scientists report effects on membrane transport of rabbit erythrocytes at power levels down to 1 mW/cm^2 for 2450 MHz microwave radiation. An attempt should be made to verify this work in the United States. At present we have been unable to verify this report but the work is still in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to carefully control the temperature of the specimen and be able to reproduce the exposure conditions. We have the capability to do this at NIEHS and can relate observed effects of microwaves to purely thermal effects.

PROPOSED COURSE: We plan to continue our study with rabbit erythrocytes and will also study other cellular systems in which sensitivity to microwaves has been reported or which are desirable as a test system for mocrowaves.

PUBLICATIONS

P.E. Hamrick, Thermal Denaturation of DNA Exposed to 2450 MHz CW Microwave Radiation, Radiation Research, 56:400-404, 1973.

P.E. Hamrick and B.T. Butler, Exposure of Bacteria to 2450 MHz Microwave Radiation, Journal of Microwave Power, 8:227-233, 1973.

Serial No.: NIEHS-EB-021
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Investigation of Non-thermal Microwave Radiation Effects on Neurological Functioning"

PREVIOUS SERIAL NUMBER: NIEHS-RSB-019

PRINCIPAL INVESTIGATORS: R.H. Wyatt, Ph.D. and G. Somjen, M.D.

OTHER INVESTIGATOR: D.I. McRee, Ph.D.

COOPERATING UNIT: Duke University, Department of Electrical Engineering, Department of Physiology

MAN YEARS

Total:	1.15
Professional:	1.10
Other:	.05

PROJECT DESCRIPTION

OBJECTIVES: The objective is to conduct an investigation into the non-thermal effects of microwave radiation on neurological functioning. Recently, efforts have been aimed at investigating possible effects on spinal reflex function in the cat.

MAJOR FINDINGS: The preliminary conclusion of the first three experiments is that irradiation of the spinal cord with microwaves of the frequency and intensity used showed no indication of an influence on synaptic transmission in the spinal cord. That energy was in fact absorbed by the tissue was demonstrated by the heating of the same. The reflex discharge typically consists of an initial "monosynapic" volley followed by an irregular "polysynaptic" discharge. The transit time through the spinal cord from the arrival of a sensory input volley to the emergence of the reflex is minimally 0.5 m/sec. Analysis of reflex amplitude, configuration and transmission time could not be related to the presence or absence of microwave irradiation at this preliminary stage.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The hazards of microwave radiation are a priority matter of current concern for NIEHS. The question of the non-thermal influence of microwaves is the principal one in the problems and consequences of the biological effects of microwaves; it is to be resolved before the degree of the hazard posed to man is determined.

PROPOSED COURSE: Continuation of the planned number of 10 to 20 experiments is in process. Also exposure to other microwave frequencies is to be pursued.

Serial No.: NIEHS-EB-022
1. Environmental Biophysics Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effects of Microwave Radiation on the Neural Response"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Donald I. McRee, Ph.D.

COOPERATING UNIT: Duke University, Department of Electrical Engineering
Department of Physiology

MAN YEARS

Total:	.1
Professional:	.1
Other:	.0

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of microwave radiation on the functioning of the central nervous system when exposed to CW and modulated microwave radiation in the frequency range of 1-10 GHz.

METHODS EMPLOYED: Isolated neurons such as the adominal ganglion of the Aplysia, the sciatic nerve of the frog, and the saphenous nerve of cats will be exposed to CW and modulated microwave radiation in the power density range of 1-10 mW/cm^2. The effects of the radiation on cell membrane potentials, action potential and conduction velocity will be investigated.

More complex systems such as the exposed spinal cord of decerebrated cats will be exposed. These test systems will be used to investigate the effects of radiation on synaptic neurotransmitters.

MAJOR FINDINGS: A review of the literature indicate that the brain and central nervous system are sensitive to microwave radiation. Many of the behavioral effects reported on animals and man result from CNS interactions. In most of the studies no explanation of the mechanism by which the microwaves interact with nerve cells is specified. Much research on low level, long term exposure is needed to determine the effects of microwave on the CNS.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of prime interest to NIEHS. The neurological and behavioral effects reported in the literature illustrates the need for significant effort in this area. At present

the accepted safe level of exposure in the U.S. is 1000 times greater than the standard in the USSR. Their standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

PROPOSED COURSE: Neural specimens will be placed in a CW or modulated microwave field. The nerves will be stimulated and the effects of the microwaves on action potential, firing potential, conduction velocity and signal transmission will be measured. If an effect is observed, the mechanism of interaction of the microwave field with the neural specimens will be investigated.

ENVIRONMENTAL MUTAGENESIS BRANCH

ENVIRONMENTAL MUTAGENESIS BRANCH
Summary Statement

The primary thrust of NIEHS' effort in mutagenesis is focused on the evaluation and definition of risks to both human germinal and somatic tissues. The Environmental Mutagenesis Branch, organized late in FY72 and anticipated to expand to full capacity within the next several years, has addressed itself to the problem by: (1) attempting to identify specific mutagenic hazards in man's environment, (2) developing new and comprehensive mutational assay systems having a high degree of relevance to human health, (3) developing a better understanding of the molecular basis of mutation in higher organisms, and (4) attempting to understand the role of repair on the process of mutation-induction.

To complement and support these efforts, the Environmental Mutagenesis Branch has continued Collaborative Research studies at the University of Wisconsin to develop an assay for determining spontaneous and induced rates of gene mutation on human somatic cells in culture. Additional studies at the Jackson Laboratory were started during the past year to develop new mouse strains for the detection of gene mutations induced in germ cells. In one project a strain will be developed for the detection of mutations which give rise to electrophoretic variants of blood serum protein and various enzymes, in the other project a new mouse strain carrying multiple chromosomal inversions is being developed to make it possible to detect a random sample of gene mutations over a greater portion of the genome to complement and extend the studies that can be made on specific genes. Both strains will enhance our ability to detect gene mutations induced in germ cells in whole mammals; the latter strain has the advantage that it should provide an assay of a much greater portion of the genome than the first strain. By Interagency Agreement we are supporting the refinement and evaluation of systems capable of detecting airborne chemical mutagens at the Brookhaven National Laboratory, and we are continuing to support the Environmental Mutagen Information Center at the Oak Ridge National Laboratory. This Center is a unique resource for the collection, storage and dissemination of information on the existence and activity of mutagens in the human environment. As part of our effort to identify specific biological hazards in our environment that may be mutagenic, a study has been started at the Institute for Medical Research to investigate and characterize virus-induced mutagenesis with special emphasis on those viruses found in vaccines. To obtain a better understanding of gene mutation at the molecular level, Miles Laboratory has been established for characterizing mutants induced by environmental chemical mutagens and carcinogens in our intramural research program. During FY74, additional efforts were made to promote expansion of programs in the Collaborative Research area which are intended to become an integral part of the Branch's research activities.

The nature of the problems in environmental mutagenesis is so broad and requires such diverse expertise and training as to far exceed what any single agency or institution could alone provide. For the development of an effective program of research in this new area of environmental health, it is essential that work in the Collaborative Research area be a continuing effort of highest priority. It is further anticipated that efforts initiated in FY74 will lead to the establishment of new contracts during FY75 in the

following areas: (1) The response of mammalian polygenic traits to chemical mutagenesis; (2) The assessment of the distribution of human mutagenic sensitivity via human cell culture; and (3) The further development and validation of rapid, automated human cytogenetic analysis.

Recruiting of personnel for a new section on Population Monitoring and Epidemiology has been successful and Dr. Charles Langley joined our staff at the beginning of FY 1974, to develop better methods to extrapolate data collected on experimental organisms to man, to develop and deploy in vivo somatic cell mutation monitoring in the human population, and to investigate population genetic variation in sensitivity to environmental agents.

We were also successful in recruiting a new head of the Somatic Cell Genetics Section and Dr. Michael Lieberman will join our staff at the beginning of FY 1975 to develop new methods for the detection of DNA repair synthesis in mammalian cells in culture.

The Environmental Mutagen Information Center has increased its data banks to 12,000 bibliographic entries. Of these approximately 11,000 have been identified with respect to agent, organism and Chemical Abstract Service Registry Number. The Center processes requests for information primarily from government agencies or universities in the United States at a rate of 1-2 per day. Wiswesser Linear Notations for compounds in the EMIC registries are being acquired and used to help bring about a better understanding of chemical-biological interactions. Also, this capability will make it possible for investigations to draw correlations between structure activity that could provide prognostic value concerning potential mutagenic activity of newly synthesized chemicals.

Within the past year, staff members of the Environmental Mutagenesis Branch have participated in the work of the new Panel on Environmental Mutagenesis and Carcinogenesis under the U.S.-Japan Cooperative Medical Science Program and organized workshops on "Long Term Toxicity of Antischistosomal Drugs" and "Mechanisms of Chemical Carcinogenesis". A similar Panel on Mutagenesis has been started with the Soviet Union as part of the US-USSR Agreement on Cooporation in the field of Environmental Protection. To foster interest and understanding of current problems in environmental mutagenesis, staff members of the Environmental Mutagenesis Branch have organized an Interagency Panel on Environmental Mutagenesis, served as members of the Mammalian Genetics Advisory Committee for the National Center for Toxicological Research and collaborated with staff members of the National Cancer Institute to set up a study on the mutagenicity of chemical carcinogens. Staff members are in communication with staff members of the Food and Drug Administration and the Atomic Energy Commission to develop complementary programs of research and to avoid unnecessary duplication of effort. Staff members have also lectured to numerous audiences in industry and at universities on problems in environmental mutagenesis. An active role has also been taken in coordinating the toxicological evaluation of hycanthone (used to treat schistosomiasis in the tropics) as a high priority Branch activity. The problem is one of unique theoretical interest as well as one of practical importance. Hycanthone is unique as a chemical mutagen in that it can produce frameshift mutations in eukaryotes, and the mechanism of mutation-induction is of great

interest. This study has involved the synthesis of new compounds by Parke-Davis and tests for mutagenicity within various sections of the Environmental Mutagenesis Branch. Antischistosomal activity is being performed in collaboration with Dr. E. Bueding at Johns Hopkins University. The collaborative study not only includes hycanthone and lucanthone, but additional structural analogs. In addition to providing basic data on structure-function relations, the study may provide clues for the development of a drug with the desirable pharmacological properties but not the undesirable toxicological properties. Staff members of the Branch have also assumed the responsibility for the coordination of the toxicological evaluation of a nitrofuran derivative, AF-2, which has been used as a food preservative in Japan since 1965. This chemical has been shown both in laboratories in Japan as well as at NIEHS to be a potent mutagen in experimental organisms. The Branch has also served as a host for numerous distinguished scientists from this country, England, Italy, Germany, The Netherlands, Japan, Belgium and Russia.

Although the Environmental Mutagenesis Branch has been organized for a brief period and a great deal of effort has gone into the remodeling of laboratory facilities, considerable progress has been made in its intramural research program.

In the Microbial and Plant Genetics Section, simple and comprehensive tests for gene mutations and other types of genetic damage are being developed in various microorganisms using Neurospora crassa. Dr. Tong-man Ong has been working on a simplified spot assay system for the detection of point mutations which will mimic in this eukaryotic organism the assay system developed by Ames for Salmonella. It is expected that this assay system will provide a short-term qualitative test for the detection of mutagenic activity which will complement the ad-3 test system. The ad-3 test system has been used by Drs. Ong and de Serres to test the mutagenic activity of various antischistosomal agents as well as various chemical carcinogens. These studies are designed primarily as structure-function analyses to determine whether it is possible to separate desirable pharmacological properties from undesirable toxicological properties.

In a new line of work Dr. Ong and Dr. Peter Matzinger will be using various mammalian and human liver microsome preparations to develop an in vitro metabolic activation system to study the mutagenicity of various chemical carcinogens in Neurospora. In another line work, Dr. de Serres is using the ad-3 test system to study mutation-induction in repair-deficient strains of Neurospora. Characterization of ad-3B mutants induced in two excision-repair deficient strains (which show enhanced mutant recovery after UV or x-irradiation) have shown that the spectra of UV-induced mutations are quantitatively different from that induced by UV in a standard wild-type strain. The enhanced recovery of ad-3 mutants in these strains can be attributed to an enhanced recovery of frameshift mutations.

Studies by Dr. Daniel Straus on Salmonella and E. coli have concentrated on the development of assay systems to detect gene duplications. In addition, various mutant strains of these organisms have been used to study the mutagenic activity of sodium azide, and various antischistosomal agents and nitrofuran derivatives. Using a new strain of diploid yeast, Dr. Majdi Shahin has studied the mutagenic

activity of various antischistosomal agents and nitrofuran derivatives on mitotic recombination and gene conversion. The simplicity of this new assay which produces pink and/or red sectors in normally white colonies makes this assay an especially useful test for genetic activity of environmental chemicals.

The ad-3 test system of Neurospora is still being used by Drs. Ong and de Serres to determine the correlation between carcinogenic and mutagenic activity. Their most recent data support previous data that chemical carcinogens produce point mutations predominantly by base-pair substitution. The ultimate objective of this work is to provide short-term tests for mutagenic activity that can predict carcinogenic activity in man.

In the Biochemical Genetics Section a variety of approaches are being used to develop new assay systems for the detection of gene mutations in mammalian somatic cells and germ cells. In a series on closely related projects new approaches are being tried to develop such assays on red blood cells that can be used directly on man. Dr. Heinrich Malling has been using both histological and immunological techniques to locate rare mutant cells in populations of normal red blood cells. Attempts to develop a staining method to detect fetal hemoglobin containing cells by contract at the University of Texas at Austin showed that a wide variety of factors other than mutation can cause a general rise in fetal hemoglobin levels in individual red blood cells. In addition, the fetal hemoglobin-specific staining technique was reliable over a very narrow range of pH. Because of these problems, the contract was terminated in December 1973. A new line of work being persued by Dr. Christopher Frantz by contract at the University of Washington in Seattle attempts to develop a method to detect rare fetal hemoglobin variants by using fluorescent antibodies. Antibodies specific to each of the two types of fetal hemoglobin $a\lambda$ or $g\lambda$ will be produced using as antigen blood from the rare individuals which have only one or the other fetal hemoglobin. The two antibodies will be conjugated with different colored fluorescent dyes. After treatment with both antisera these rare cells containing only one antibody will fluoresce in only one color, whereas normal red blood cells will fluoresce in both colors.

Another approach towards the detection of point mutations in mammalians has been to use electrophoresis to detect specific enzyme mutations that result in either a non-functional protein or a functional protein with an altered electrical charge. Two inbred mouse strains are being used which differ with respect to nine enzyme loci to determine the sensitivity of this assay an experiment is being performed in which adult males were irradiated with 1000 r gamma rays from a ^{60}Co source. The sensitivity of this assay system is being increased by Dr. Valcovic in a contract with the Jackson Laboratory in which the number of loci which can be analyzed will be increased from 9 to about 22. In a similar line of work, Dr. Malling is looking for electrophoretic variants of any blood serum potential in 10 different strains of inbred mice.

New work has been started during FY74 to develop methods for measuring the frequency of point mutations in male germ cells, especially mature sperm. This work has involved the use of histochemical staining techniques to detect the presence of specific enzymes. Cells which stain under selective conditions would be presumptive mutants and would provide a direct measure of mutational events.

Considerable progress in this system has been made on the refinement of *in vitro* assay systems for metabolic activation using a supernatant of mouse liver homogenate or a purified microsomal fraction from mouse liver. These extracts have been used in coordination with *S. typhimurium* strain G46 to determine the optimal activation conditions for dimethylnitrosamine (DMN). Two agents (3-methylcholanthrene and phenobarbital) which enhance DMN activation. In other studies marked strain differences were found between different strains of inbred mice with regard to activation.

One of the most exciting developments in this section, with regard to new and promising assay systems is the work of Dr. George Hoffmann. He has developed a new simplified system for the detection of forward-mutations at a single locus (*aza-3*). Treated conidia are plated in the presence of 8-azaguanine and in a heterokaryon heterozygous for *aza-3*, increased frequency of mutants were found after treatment with a variety of agents. This new assay system should be able to detect a broad range of genetic alterations ranging from base-pair substitutions to chromosomal deletions and should provide a simplified assay for the detection of the mutagenic activity of environmental chemical mutagens.

Work in the Somatic Cell Genetics Section has concentrated on the validation of the specific locus assay in mouse lymphoma cells. In the experiments of Dr. Donald Clive this new assay system has been used to evaluate the mutagenicity of various structurally related antischistosomal drugs. This is basically a structure-activity study with primary emphasis on determining whether desirable pharmacological properties can be dissassociated from undesirable toxicological properties with slight modifications in basic structure. With this approach several analogs of the potent mutagen, hycanthone, have been identified which are potentially more useful than hycanthone for chemotherapy.

In collaboration with Dr. Peter Voytek in the Environmental Toxicology Branch, Dr. Clive has begun the biochemical characterization of the thymidine kinase enzymes in various forward-mutations as well as reverse-mutations to determine the basis for mutation-induction at this locus at the molecular level. Studies on various revertant clones supports the hypothesis that structural gene mutations are being detected at the thymidine kinase locus after mutagenic treatment and that the mutation rates observed are a direct indication of the induction rates of this type of genetic damage. In a closely related line of work Dr. James Patterson, in collaboration with Dr. Clive is determining the quantitative relationship between the number of lesions induced in DNA and the number of mutations observed after exposure to a given mutagenic agent. Comparison of the effects of three different alkylating agents has shown that quantitative relationship is mutagen-dependent.

During the past year studies in the Mammalian Genetics Section have focused on the comparative sensitivities of different mouse strains as well as different routes of injection. In one project, Dr. William Sheridan is comparing the induction of dominant lethal mutations induced in a wide variety of inbred strains of mice after treatment with a given dose of ethyl methanesulfonate (EMS). The objective of this work is to develop a

battery of tester strains ranging from very sensitive to very resistant which will better mimic the range of variation that we can expect in the heterogeneous human population. In a closely related line or work, Dr. Eugene Soares, is comparing the effect of different routes of ingestion of EMS in two inbred strains and one hybrid strain. A comparison of the levels of dominant lethality found will be performed to determine the optimal conditions for the detection of the genetic activity of this compound.

In another line of work Dr. Sheridan is studying methods for enhancing the sensitivity of the detection of genetic damage to spermatogonial stem cells of mice. Knowledge of the degree of sensitivity of stem cells to mutation-induction after exposure to a given mutagen is an essential prerequisite for benefit-risk evaluation, since damage to the cells persists throughout the reproductive life of an individual.

In a new contract effort with the Jackson Laboratory, Dr. Sheridan is attempting to develop a new assay system in mice for the detection of gene mutations. The approach being used is to develop a strain carrying multiple inversions. In theory it should be possible to develop a system in mice similar to one that has been used successfully to detect gene mutations in Drosophila. Such an assay makes it possible to look for mutagenic effects on a more or less random population of genes to complement the studies which we can make on effects at specific loci.

During the past year, Dr. Charles Langley has joined the staff and begun the development of an intramural program of the research in the Population Genetics and Epidemiology Section. In one line of work in collaboration with statisticians in the Environmental Biometry Branch and geneticists at North Carolina State University, Dr. Langley is analyzing gene frequency changes in response to exposure to various environmental chemicals. These experiments involve monitoring gene frequencies of experimental Drosophila populations as they are exposed to various toxic environmental agents. In another line of work, experiments are being performed to study the inactivation kinetics of human lymphocytes to try to develop a simple assay that can be used in population monitoring to estimate the variability in response that might be obtained after exposure to a given mutagenic agent.

In conclusion, during the past year we have been successful in recruiting key personnel for our intramural program, have started cooperative programs with other Branches in the Institute, have coordinated program development of the Branch with similar programs in other Federal agencies and have established lines of communication with newly developing scientific organizations for environmental mutagenesis abroad. Along with the organization of workshops and conferences, these approaches will be used to stimulate research and develop in this rapidly developing area of environmental health science.

Serial No: NIEHS-EM-001
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Detection of Chemical Mutagens with Salmonella typhimurium"

PREVIOUS SERIAL NUMBER: NIEHS-MB-004

PRINCIPAL INVESTIGATOR: Daniel S. Straus, Ph.D.

OTHER INVESTIGATORS: Martha Brown

COOPERATING UNITS: Professor Bruce N. Ames
Department of Biochemistry
University of California
Berkeley, California 94720

Professor Philip E. Hartman
Department of Biology
Johns Hopkins University
Baltimore, Maryland 21218

MAN YEARS

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: The general objective of this project is to work on further refinements in the Salmonella typhimurium reverse mutation system developed by Ames, Hartman and coworkers. In particular, it is of interest to identify new strains for expanding the spectrum of mutations detected by the system, so that ultimately all types of base-pair interchange and frameshift mutations are detected.

The reverse mutation system is also being used to study the mutagenic activity of some specific compounds of environmental and pharmacological interest. Particular attention has been focused on the study of a series of thioxanthene compounds related in structure to the antischistosomal drug hycanthone. The mutagenic specificity and mechanism of action of sodium azide are also being investigated.

METHODS EMPLOYED: The effect of chemicals on the reversion frequency of histidine-requiring bacteria is quantitated either directly on plates, or after treatment of cells in suspension.

MAJOR FINDINGS: (1) Construction of recA tester strains: A series of four Salmonella tester strains (TS24, TS25, TS26 and TS27) have been constructed containing the recA1 mutation with hisG46, hisC207, hisC3076 and hisD3052. These strains will be useful in identifying agents that induce mutations via the recA and lex-dependent "misrepair" pathway.

(2) Mutagenic specificity of sodium azide in bacteria: Sodium azide has been shown to revert hisG46 among the Ames tester strains, but none of the frameshift testers. Azide is a much more potent mutagen in strains containing deletions of the uvrB gene (TA1535 and TA1950) than in strains with wild-type repair. It is therefore concluded that the damage to DNA caused by azide is very efficiently repaired by the excision repair pathway. A strain containing hisG46 in combination with the recA1 gene (TS24) is as sensitive to azide mutagenesis as rec^+ strains. This indicates that azide mutagenesis does not involve recA-dependent misrepair.

We have observed that azide produces an extremely volatile mutagen under the conditions of the bacterial plate test plate (37C, low pH). This result, as well as the pH dependence for azide mutagenesis observed by others, indicates that the probable active mutagen is the protonated form of azide -- hydrazoic acid. (Hydrazoic acid has a pK of 3.72 and a boiling point of 37C.) The observation that azide produces a volatile mutagen suggests that follow-up studies of industrial workers with symptomatic hydrazoic acid intoxication may be of future interest. The volatility of hydrazoic indicates that azide should be handled in the laboratory with extreme caution.

(3) hisC117 as a new Salmonella tester strain: A number of laboratories have reported that the Japanese nitrofuran food additive AF-2 does not revert the four Ames tester strains, although it is a strong mutagen in other systems. A Salmonella ochre mutant (hisC117) has been found that is reverted by AF-2. Preliminary experiments suggest that this strain may have a reversion specificity similar to an E. coli ochre mutant WP2 trp, studied in detail by Witkin, Bridges and others. Because these two strains have a different specificity than the four Ames tester strains, it will be useful to add one of them to the set.

(4) Drugs in the hycanthone series with reduced mutagenicity: In collaboration with Dr. P. Hartman and Dr. E. Bueding (Johns Hopkins), studies are continuing on the mutagenicity of drugs in the hycanthone series. One extremely promising group of compounds has been identified: compounds containing an alkyl substitution in the proximal nitrogen on the side chain. One of these compounds, containing a piperazyl modification of the side chain and a chloro substitution in the 6-position of the thioxanthene ring system, has less than 1% of the mutagenic activity as hycanthone, and also a much lower toxicity in mammals. From space-filling models, it can be seen that the piperazyl side chain modification restricts rotation of the side chain, and this could be the explanation for the lowered mutagenic activity. This series holds some promise as an improvement to hycanthone for chemotherapy in humans.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Among the various mutagen testing assays, the bacterial reverse mutation test has the advantages of speed, simplicity, and sensitivity. The reverse mutation

test can also in many cases provide detailed information concerning the molecular mechanism of mutagenesis by chemicals. Further development of this test system will therefore be of great value in the area of environmental mutagenesis.

PROPOSED COURSE: In collaboration with Ames and Hartman (cooperating units above) an attempt is being made to expand the set of strains used in the bacterial reverse mutation system to detect more frameshift and base-pair interchange mutagens.

In addition, attention will continue to be directed toward using this system for testing various compounds of environmental and pharmacological interest.

Serial No: NIEHS-EM-002
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Detection of Gene Duplications in Escherichia coli and Salmonella typhimurium"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Daniel S. Straus, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.7
Professional:	0.7
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: Genetic aberrations involving the rearrangement of long segments of DNA, such as deletions, translocations, inversions, and duplications, are relatively poorly understood in bacteria because of the difficulty in analyzing many of these events genetically. These events have all been grouped together under the title "illegitimate recombination" because they all seem to involve the joining of unrelated, or not obviously related, DNA sequences.

Although some mutagens are known to induce deletions in bacteria, little is known about the effect of mutagens of the frequency of other genetic events arising from aberrant recombination. This project has been directed toward a study of the effect of mutagens in inducing duplications in the glyS region of the E. coli and S. typhimurium chromosomes. It is hoped that this study will provide new information concerning the mechanism by which aberrant recombination occurs in bacteria, and also provide new test systems for analyzing the effect of mutagens on various types of aberrant recombination.

METHODS EMPLOYED: Tandem gene duplications have been selected in the glyS region of the E. coli chromosome using strain BF87 previously isolated and described by Folk and Berg (J. Molec. Biol., 58: 595-610). A simplified method has been devised for identifying strains containing duplications of glyS. The method involves serial single colony isolations on tryptone glucose extract medium.

A group of S. typhimurium glyS mutants have been isolated following mutagenesis with UV or 2-aminopurine, and penicillin enrichment. These strains are being used in a study of the dependence of duplication formation on the recA-mediated general recombination system.

A new method for selection duplications in S. typhimurium has been devised. The method involves selection for mutants with fast growth on l-malate as sole carbon source.

MAJOR FINDINGS: (1) Effect of mutagens on the frequency of tandem gene duplications. Four mutagens (ultraviolet light, nitrous acid, the acridine half-mustard ICR-372, and niridazole) have been found to increase the frequency of tandem gene duplications in the glyS region of the E. coli chromosome. This result has been obtained by quantitating the spontaneous and mutagen-induced reversion frequency of a glycyl-tRNA synthetase mutant of E. coli. Following mutagenesis, as many as 0.2% of the survivors were observed to contain tandem duplications in the glyS region. In addition, at least three classes of stable revertants of the glyS mutant have been identified, including strains which are indirectly suppressed (owing to a block in glycine deamination or transamination); strains that appear to contain intragenic suppressors of the glyS mutation, and strains which contain other types of suppressors, or possibly, non-tandem duplications of glyS.

The observation that all four mutagens are active in inducing duplications suggests that the stimulation is due to some type of DNA damage (or DNA repair intermediate) common to a variety of mutagenic treatments, such as single strand breaks and gaps. Such breaks could act as precursors for the "recombinational" intermediates involved in the formation of duplications. Interestingly, some mutagens appeared to give a much higher proporation of duplications (compared to stable suppressor mutations) than others. This might reflect a different efficiency of various mutagens in increasing the frequency of aberrant recombination versus gene mutations. The strong mutagenic activity observed for niridazole in bacteria indicates that this compound should be tested further for mutagenicity and carcinogenicity.

(2) Investigations of the mechanism of tandem gene duplications: Preliminary experiments on the mechanism of tandem gene duplications in S. typhimurium suggest that the process is recA-dependent. Among the various possible mechanisms by which duplications could arise, those involving physical breakage and reunion of DNA are most consistent with this observation.

(3) New method for selecting duplications: S. typhimurium grows extremely slowly on l-malate as sole source of carbon. Fast-growing mutants appear at very high frequencies when S. typhimurium is plated on l-malate. One type of fast grower segregates malate slow growers at a very high frequency, which suggests that the fast growers may contain chromosomal duplications. The genetic basis of this phenomenon is currently under study.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Highly refined techniques currently exist for the detection of point mutations in bacteria. It would be extremely valuable to have other simple systems for detecting genetic events involving aberrant recombination. Hopefully, a

deeper understanding of how aberrant recombination occurs in prokaryotes will provide suggestions concerning the mechanism by which rearrangements occur in eukaryotic chromosomes.

PROPOSED COURSE: Further studies will be directed toward a better understanding of the mechanism by which tandem gene duplications arise in bacteria. Of particular interest is the preliminary evidence suggesting an involvement of recA.

Experiments are also currently in progress to find out more about the nature of the duplications that occur after selection on l-malate.

Serial No: NIEHS-EM-003
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Mutation-Induction in Repair-Deficient Strains of Neurospora crassa"

PREVIOUS SERIAL NUMBER: NIEHS-MB-002

PRINCIPAL INVESTIGATOR: Frederick J. de Serres, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Miles Laboratory

MAN YEARS

Total:	1.6
Professional:	0.6
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Recent studies have shown that the process of gene mutation is under genetic control, and marked quantitative differences have been observed in mutant strains. In bacteria, repair-deficient strains have been found to act as mutators; and in yeast additional mutants have been observed to have the same properties. Mutant strains which show a higher level of response after mutagen treatment would be especially useful in screening programs to detect genetic activity resulting from low levels of exposure on weak mutagens. Exploratory experiments of our own have shown that repair-deficient haploid strains of Neurospora have marked quantitative and qualitative effects on the induction of point mutations at the ad-3A and ad-3B loci. The ad-3 test system is unique in that the mutations can be thoroughly characterized, including a presumptive identification of the genetic alterations in the point mutations at the molecular level. All possible effects were found including a strain which is completely resistant to mutation-induction after UV irradiation. In two repair-deficient strains, upr-1 and uvs-2 enhanced mutant recovery was found after UV irradiation. Genetic characterization of the ad-3 mutants recovered in these experiments will make it possible to determine whether the quantitative differences are due to qualitative differences in the spectrum of genetic alterations at the molecular level.

METHODS EMPLOYED: Ad-3 mutants from forward-mutation experiments on upr-1 and uvs-2 were characterized by means of heterokaryon tests for complementation. These tests distinguish ad-3A mutants from ad-3B mutants and determine

the complementation patterns of ad-3B mutants showing allelic complementation.

MAJOR FINDINGS: The genetic characterization of UV-induced ad-3B mutants in the upr-1 and uvs-2 strains have complementation patterns which are significantly different from UV-induced ad-3B mutants induced in a standard wild-type strain. In fact, the spectra of complementation patterns in these two strains is exactly like that shown by ICR-170 (a frameshift mutagen) rather than UV.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The present data strongly show that excision-repair deficient strains are more sensitive indicators of genetic activity than standard wild-type strains after UV- or x-irradiation. They also indicate that the spectrum of genetic alterations produced by UV is qualitatively different from that found in wild-type strains. Since excision-repair deficient strains of bacteria are being used by others to characterize the spectrum of genetic alterations shown by carcinogens and environmental mutagens, our present data suggest that such characterization may be in error. The shift in the sprectrum of complementation patterns found after UV in the upr-1 and uvs-2 strains can be best explained on the basis of an enhanced recovery of mutants due to frameshift mutations. If this is a general phenomenon, the use of repair-deficient strains could result in the erroneous classification of mutagens as frameshift mutagens.

PROPOSED COURSE: In one line of work we will characterize the ad-3B mutants induced by x-rays in the upr-1 and uvs-2 strains to compare with x-ray-induced ad-3B mutants induced in a standard wild-type strains. In a second line of work we will induce ad-3 mutants in the upr-1 strain with various chemical carcinogens. The spectrum of complementation patterns induced by these carcinogens in the upr-1 strain will be compared with those induced by the same carcinogens in a standard wild-type strain.

Serial No: NIEHS-EM-004
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Mutagenicity and Mutagenic Specificity of Environmental Mutagens in Neurospora crassa"

PREVIOUS SERIAL NUMBER: NIEHS-MB-003

PRINCIPAL INVESTIGATOR: Tong-man Ong, Ph.D.

OTHER INVESTIGATORS: Frederick J. de Serres, Ph.D.

COOPERATING UNITS: Miles Laboratory, Elkhart, Indiana

MAN YEARS

Total:	1.6
Professional:	0.6
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: During the past 35 years, thousands of nitrofurans have been synthesized. Many of these compounds are being used as chemotherapeutic agents in the treatment of disease in man. Some other nitrofurans are being used as antibacterial food additives and preservatives. The biological activity of nitrofurans, therefore, is very closely related to human health. Little is known, however, about their teratogenic, carcinogenic and mutagenic effects. Studies on the antibacterial and antischistosomal activities have shown that the nitro-group at the C5 position of the furan ring and one of the many variations of the side chain at the C2 position are essential factors for such activities. It has also been shown that the nitro-group at the C5 position plays a major role in the carcinogenic activity of nitrofurans. It would be of interest to determine whether some of the known nitrofurans are mutagenic in Neurospora crassa and to determine whether there is any relationship between mutagenicity and chemical structures of nitrofurans.

METHODS EMPLOYED: The ad-3 test system of N. crassa developed by F.J. de Serres is used in this study. In general, mutation induction experiments could be described as follows: conidia from a genetically marked two-component heterokaryon are harvested and are treated with different concentrations of nitrofurans in sodium-phosphate buffer, pH7, at 30°C for 2 hours in a water bath shaker in the dark. At the end of the treatment, chemicals are decanted and conidia are washed by centrifugation. The treated and untreated conidia are inoculated into 10 liters of Westergaard's medium with the appropriate supple-

ments. After 7 days incubation in the dark at 30°C the frequency of purple ad-3 mutants are determined in each treatment. The isolated ad-3 mutants are then characterized by a series of genetic tests.

MAJOR FINDINGS: The mutagenicity of nitrofurazone, a commonly used antibacterial drug; AF-2, a widely used antibacterial food additive in Japan; SQ 18,506, an antischistosomal agent; FANFT, a potent carcinogen nitrofuran; and six other closely related compounds were studied in N. crassa. The results show that AF-2, SQ 18,506, and FANFT are potent mutagens. The ad-3 mutation frequencies increased more than 100-fold over the spontaneous frequency after the conidia were treated with a 0.1 mM solution of any of these three compounds. On a per mole basis, the mutagenicity of these compounds in N. crassa is higher than those of many well known chemical mutagens such as nitrous acid, ethylmethanesulfonate, methylmethanesulfonate, hydroxylamine, ethylenimine and hycanthone. Nitrofurazone is also mutagenic; however, this compound appears to be a weak mutagen in N. crassa. The remaining six compounds are not mutagenic under the conditions tested. These data indicate that the highly mutagenic properties of AF-2, SQ 18,506, and FANFT are attributable to two structural features: (1) a NO_2 group at the C5 position, and (2) a particular side chain at the C2 position. The side chain at the C2 position required for mutagenic activity seems to be more specific than those required for antibacterial and antischistosomal activities.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many nitrofurans are used as antibacterial drugs or as antibacterial food additives or food preservatives. Studies on the mutagenic activities of nitrofurans could provide information in regard to the potential mutagenic hazards of nitrofurans in humans. Furthermore, studies on the mutagenicity and the chemical structure relationships of nitrofurans could lead to a better understanding of the mutagenicity and other biological activities of nitrofurans such as antibacterial, antischistosomal and carcinogen activities and might lead to a production of non-mutagenic compounds that could be used as food additives or as drugs for the treatment of disease in man.

PROPOSED COURSE: Future studies on the mutagenicity and mutagenic specificity of environmental agents and other nitrofurans will be focused on those agents that are closely related to human health. Future studies will also be carried out to determine types of genetic alterations caused by environmental agents, and to determine the relationship between their mutagenic activities and their chemical structure.

Serial No: NIEHS-EM-005
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Spectrum of Genetic Alterations Caused by Chemical Carcinogens in Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Tong-man Ong, Ph.D.

OTHER INVESTIGATORS: Frederick J. de Serres, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: Many environmental agents and synthetic chemicals are carcinogenic in man and other mammals. The mechanism by which these agents cause cancer is not yet understood, however, the somatic mutation theory is one of the most attractive hypotheses for the origin of neoplasia. Recent studies on the mutagenic activity of chemical carcinogens have shown that chemical carcinogens induce frameshift mutations in Salmonella typhimurium but induce chromosomal deletions in Drosophila melanogaster. Our previous studies have shown that there is a positive correlation between carcinogenicity in laboratory mammals and mutagenicity in Neurospora crassa among those carcinogenic compounds tested. It would be of interest to determine whether chemical carcinogens induce a particular type of genetic alteration in N. crassa.

METHODS EMPLOYED: The ad-3 test system of N. crassa developed by F.J. de Serres is used in this study. With this system one can isolate ad-3 mutants on N. crassa and determine the types of genetic alterations at the molecular level (chromosomal deletions). In general, the ad-3 mutants isolated from forward mutation experiments are made homokaryotic for the adenine requirement. The genotype and the complementation pattern of each mutant is determined by heterokaryon tests with various strains used as testers. Mutants were then tested by means of dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS: Ad-3 mutants induced by the chemical carcinogens, 1-phenyl-3-monomethyltriazene (PMMT), 1-phenyl-3, 3-dimethyltriazene (PDMT), 4-nitroquinoline 1-oxide (4NQO), 1,2,4,5-diepoxypentane (1,2,4,5-DEP), 1,2,7,8-diepoxyoxtane (1,2,7,8-DEO), ethylenimine, 2-naphthylamine (2-AN) and N-hydroxy-1-naphthylamine were characterized genetically by dikaryon, trikaryon and complementation tests. The results, in general, showed that closely related compounds except PDMT and DMMT, cause similar spectra of genetic alterations in N. crassa and that the ad-3B mutants induced by potent chemical carcinogens such as 4NQO, 4HAQO, PMMT, aflatoxins and 2-AN have relatively high frequencies of leakiness, allelic complementation and nonpolarized complementation patterns. It seems, therefore, that potent chemical carcinogens cause predominantly base-pair substitution mutations in eukaryotic organisms.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of chemical carcinogens in N. crassa might provide a system that could be used for screening carcinogenic chemical agents in the human environment. Furthermore, the studies on the spectrum of genetic alterations, caused by chemical carcinogens in N. crassa might provide information in regard to the relationship between carcinogenesis and mutagenesis of chemical agents.

PROPOSED COURSE: Future study will be devoted to those chemical carcinogens in which metabolic activation is necessary for the expression of their carcinogenic activities.

PUBLICATIONS

Ong, T. and de Serres, F. J.: Mutagenic activity of ethylanimine in Neurospora crassa. Mutation Research 20: 251-258, 1973.

Ong, T. and de Serres, F. J.: Genetic characterization of ad-3 mutants induced by chemical carcinogens, 1-phenyl-3-monomethyl-triazene and 1-pheno-3,3-dimethyl-triazene, in Neurospora crassa. Mutation Research 20: 17-23, 1973.

Serial No: NIEHS-EM-006
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Development of a Spot Test System in Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Tong-man Ong, Ph.D.

OTHER INVESTIGATORS: Marion Blois

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The adenine-3 (ad-3) forward mutation test system in Neurospora crassa developed by F. J. de Serres is one of the best systems in studying chemical and physical mutagenesis in eukaryotic microorganisms. With this test system, recessive lethal mutations resulting from either chromosome deletions or point mutations can be recovered at the ad-3 region. The isolated mutants can be characterized by a series of genetic tests and the type of genetic alterations at the molecular level can be determined. The limitations of this test system, however, are that one cannot screen for the mutagenicity of a large number of chemicals in a short period of time and that not every laboratory can use this test system. It seems, therefore, necessary to develop a spot test system in N. crassa and to search for different tester strains which can be used for detecting specific types of genetic alterations such as base-pair substitutions and frameshift mutations. The development of a spot test in N. crassa will make the ad-3 test system a more complete and useful system for the detection and evaluation of the mutagenic activity of environmental agents.

METHODS EMPLOYED: Development of a spot test system will involve two steps. The first step will be establishing the optimal test conditions in N. crassa and the second step will be finding the tester strains which can detect several different types of genetic alterations. Conidia from 7 day-old vegetative cultures of ad-3 mutants will be harvested. A specific number of conidia will be added into 2 ml of cover medium (Fries' minimal medium). The mixture of conidia and cover medium will be poured onto a plate containing 20 ml of Fries' minimal

medium. Samples of known chemical mutagens will be placed on the center of the plate. After 6 days incubation at 30°C revertants from each plate will be recorded. The results from different test conditions will be compared. The conditions that give optimal results will be used for the future spot tests.

In the second step, reverse mutations of a large number of ad-3 mutants from different mutagenic origins will be tested with several known chemical mutagens (MNNG, Hydroxylamine, 4NQO, ICR-170, AF-2, EMS, etc.) by spot tests. Mutants which exhibit the best responses to the mutagens will be selected as tester strains.

MAJOR FINDINGS: Preliminary studies have shown that the spot test can be used in N. crassa.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a spot test system will provide a simple, economical and quick system in eukaryotes for the detection and evaluation of the mutagenic activity of chemical agents in the human environment.

PROPOSED COURSE: Future efforts will be concentrated on the development of a complete tester set which will include strains that can detect different copies of base-pair substitution mutations as well as frameshift mutations.

Serial No: NIEHS-EM-007
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Mutagenic Activity of Antischistosomal Drugs and Their Analogs in Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Tong-man Ong, Ph.D.

OTHER INVESTIGATOR: Frederick J. de Serres, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: Hycanthone, lucanthone and niridazole, chemotherapeutic agents in the treatment of Schistosomiasis, are attracting wide concern because of their potential carcinogenic and mutagenic hazards to treated individuals in the human population. Our previous studies have shown that hycanthone, lucanthone and three of their indazole analogs (IA-3, IA-4 and IA-5) are mutagenic in N. crassa. It would be of interest to determine whether the other antischistosomal agents, niridazole and IA-6 (indazole analog of hycanthone) are mutagenic in N. crassa and to determine the type of genetic alterations caused by hycanthone, lucanthone and their indazole analogs.

METHODS EMPLOYED: The ad-3 test system of N. crassa is used in these studies. Conidia from a genetically marked two-component heterokaryon are harvested and are treated with different concentrations of antischistosomal agent in sodium-phosphate buffer, pH 7, at 30°C for 2 hours in a water bath shaker in the dark. At the end of the treatment chemicals are decanted and conidia are washed by centrifugation. The treated and untreated conidia are inoculated into 10 liters of Westergarrd's medium with the appropriate supplements. After 7 days incubation in the dark at 30°C, the frequency of purple ad-3 mutants are determined in each treatment. The isolated mutants are made homokaryotic for the adenine requirements. The genotype and the complementation patterns of each mutant are determined by growing each mutant with each complementation tester. Based on the complementation patterns, mutants can be classified as noncomplementing, polarized complementing and nonpolarized complementing mutants. The mutants are further tested with dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS: IA-6 is mutagenic in N. crassa whereas niridazole is not mutagenic under the conditions tested. On a per mole basis, IA-6 is more mutagenic and toxic than hycanthone. IA-6 however, is less toxic and mutagenic than lucanthone, IA-3, IA-4 or IA-5.

Ad-3 mutants induced by hycanthone, lucanthone, IA-3, IA-5 and IA-5 are being made homokaryotic for the adenine requirement and will be characterized by a series of genetic tests.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of niridazole, hycanthone, lucanthone and their structural analogs are essential and important, since hycanthone and niridazole are widely used for treatment of Schistosomiasis in the human population. It is important to determine the potential mutagenic hazard of these compounds to the treated individuals. Studies on the relationship between structures and mutagenic activities of hycanthone and its analogs might lead to a production of non-mutagenic compounds that can be used for the treatment of Schistosomiasis. This analysis will provide some experience with regard to structure-function relationships which will be important for future work with mutagenic environmental chemicals. We need to know whether we can develop structural analogs of desirable, but mutagenic chemicals that will retain the desired pharmacological properties, but not the undesirable mutagenic properties.

PROPOSED COURSE: Future studies on the mutagenic activity of hycanthone and its analogs will be focused on the mechanism of mutation induction by these compounds in N. crassa.

Serial No: NIEHS-EM-008
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Use of a Liver Microsome Activating System for the Detection of Mutagenic Effects of Chemical Carcinogens in Neurospora crassa"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Peter K. Matzinger, Ph.D.

OTHER INVESTIGATORS: Tong-man Ong, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.4
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this investigation is to develop methods which could detect the mutagenic effects of chemical carcinogens such as aflatoxins in Neurospora crassa. Aflatoxins are metabolites of the fungus Aspergillus flavus which contaminates various animal and human foodstuffs, e.g. peanuts. As it has been established that aflatoxins are some of the most powerful chemical carcinogens, it is clearly visible that they are dangerous for human beings and are suspected of being a primary cause of human liver cancer in certain areas.

There is evidence that metabolic activation of aflatoxins is necessary for their carcinogenic effect. In Neurospora crassa it has been shown that a direct treatment of conidia with aflatoxin does not increase the mutation frequency whereas such an increase is observed in vegetative growing cultures. A liver microsome activating system for aflatoxins has been used with bacteria for detecting the mutation frequency. It would therefore, be of considerable interest whether such a system with hamster liver microsomes could be used with Neurospora crassa as a tester organism.

METHODS EMPLOYED: Conidias are harvested from a vegetative culture of heterokaryotic N. crassa and incubated in vitro together with the following: (1) liver microsomal preparations, (2) a NADPH generating system consisting in glucose-6-phosphate, NADP, and glucose-6-phosphate dehydrogenase, and (3) aflatoxin B_1.

Incubation temperature is 37°C, according to the body temperature of the microsome donor. After the treatment the survival of conidia is determined. Samples of the treated and untreated conidia are incubated in fresh medium. The mutation frequency can be determined by using the ad-3 forward mutation system of N. crassa.

MAJOR FINDINGS: The project was initiated in March 1974.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It should be possible to establish an in vitro microsome activation technique in Neurospora crassa which could be used as a test system for chemical agents which need liver microsome activation. Furthermore this study could provide the information in regard to the biological activity of aflatoxins.

PROPOSED COURSE: Active studies will be extended to human liver microsomes in order to get direct evidence that certain chemical substances are metabolized by human liver enzymes into highly carcinogenic and mutagenic compounds.

Serial No: NIEHS-EM-009
1. Environmental Mutagenesis Branch
2. Microbial and Plant Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Development of a Screening System for Mitotic Recombination and Gene Conversion in Yeast in the Absence and Presence of Metabolic Activation"

PREVIOUS SERIAL NUMBER: NIEHS-MB-005

PRINCIPAL INVESTIGATOR: Majdi M. Shahin, Ph.D.

OTHER INVESTIGATORS: Frederick J. de Serres, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	1.2
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: The purpose of the present project is to develop a screening system for the detection of mitotic recombination and mitotic gene conversion in the absence and presence of metabolic activation. Genetic alterations can occur by either mutations or recombination. Mitotic recombination is a reciprocal process resulting from exchanges between non-sister chromatids of homologous chromosomes. Mitotic gene conversion is a process characterized by its failure to yield reciprocal products during recombination. Both events can create a serious type of genetic change in diploid organisms, because their occurrence can cause harmful recessive genes to become homozygous and thereby permit their phenotypic expression. Among eukaryotes, Saccharomyces cerevisiae diploid strains are convenient organisms in which recombination can be studied after treatment with chemical or physical agents and related to man.

METHODS EMPLOYED: Mitotic recombination could be determined by using the yeast strain D5. Strain D5 carries two phenotypically distingquishable ad-2 alleles which complement each other on minimal medium so that the diploid heterozygote forms white colonies. Genetic activity is indicated in strain D5 by the appearance of six phenotypically distinguishable colony types. The induction of gene conversion could be studied by using strain D4. It requires both adenine and tryptophan for growth. Gene conversion at the ad-2 or try-5 loci produces cells capable of growth on medium deficient in either adenine or tryptophan.

MAJOR FINDINGS: A number of compounds including the antischistosomal agents hycanthone methanesulfonate, lucanthone, SQ 18,506 as well as the antimicrobial food additive, furylfuramide were tested for genetic activity in yeast. All four compounds were found to be genetically active in strain D5 system (mitotic recombination system). In a detailed study with hycanthone and SQ 18,507, hycanthone failed to induce gene conversion in strain D4; was very toxic to both strains at pH 7.2 but far less toxic at pH 5.5. SQ 18,506 on the other hand showed no pH dependence, and at both pH's used, rapid increases in the frequencies of convertants at both the ad-2 and try-5 loci were found. This means that SQ 18,506 is genetically more active than hycanthone, whereas hycanthone is much more toxic at the higher pH. It should however, be pointed out that when hycanthone was used, the frequency of red and pink sectored colonies was higher at pH 5.5 than at 7.2. These observations indicate that hycanthone is genetically active and that its mode of action is pH dependent.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lack of specificity, the simplicity, the rapidness and the inexpensiveness of both systems are the evidence that strains D4 and D5 can be considered as ideal diploid organisms to study the induction of mitotic recombination and gene conversion, the two possible alternatives of the genetic material other than mutations. Further, that both systems can be used as a tool for detection of potential agents to induce genetic damage.

PROPOSED COURSE: Future efforts will be made to compare hycanthone, lucanthone and structural analogs; as well as SQ 18,506, furylfuramide and niridazole. Some of the properties of the two components of D5 (haploid strains: AZ-5A and 4-12D) will be explored. New suspected agents will be tested. In addition, the two strains will be used occasionally in host-mediated assay and microsomal test systems.

PUBLICATIONS

Shahin, M. M. and Kilbey, B. J.: Genetic activity of the antischistosomal agent SQ 18,506 in yeast. Mutation Research (in press).

Shahin, M. M. and de Serres, F. J.: The effect of pH on hycanthone methanesulfonate induced inactivation and mitotic recombination in Saccharomyces. Mutation Research (in press).

Serial No: NIEHS-EM-010
1. Environmental Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Electrophoretic Mobility Mutation Analysis in Mammals"

PREVIOUS SERIAL NUMBER: NIEHS-MB-007

PRINCIPAL INVESTIGATOR: Lawrence R. Valcovic, Ph.D.

OTHER INVESTIGATORS: Heinrich V. Malling, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	4.4
Professional:	1.4
Other:	3.0

PROJECT DESCRIPTION

OBJECTIVES: Classically, the detection of point mutations in mammals has relied upon the induction of visible recessive mutations. These studies have not yet identified the molecular event responsible for producing the mutant phenotype. However, the study of specific enzyme mutations using electrophoresis has the capability of detecting mutations that result in a non-functional protein or a functional protein with altered electrical charge. Our objective is to develop a single mammalian test system that will detect both electrophoretic mobility (EPM) and inactive enzyme (IAE) mutations in the same loci. This will enable us to determine the relative frequencies of these two types of mutational events in response to various types of mutagenic agents.

METHODS EMPLOYED: The ratio between mutations resulting in IAE-mutations and EPM-mutations can be estimated from the changes in the zymogram of heterozygous offspring from a cross between two inbred strains which differ in the electrophoretic patterns of several enzymes. In the simplest case let us assume the normal heterozygote will have three bands for a particular enzyme. A mutant induced in either parent can result in one of three different electrophoretic patterns: (a) the presence of any one band indicating that the mutant is inactive; (b) the presence of any two bands, one at the normal position for one of the parents, and the other at any position indicating that the inactive enzyme will form an active aggregate with the wild-type enzyme; and (c) the presence of three bands, two with changed positions, indicating an EPM-mutation.

Since case (a) and (b) indicate inactive enzymes and (c) an EPM-enzyme, a direct estimation of the ratio between IAE-mutations and EPM-mutations can be made.

We have selected two inbred mouse strains which differ with respect to nine enzyme loci. Additional non-variant bands will be apparent with some of the strains; and while the number of loci that these represent is unknown, EPM-mutations may be detected at these positions.

Since it is important to correlate this system with Russell's specific locus test in mice, the first mutagen treatment utilized was ionizing radiation. Adult males were irradiated and after a sterile period ranging from 4 to 11 months they were mated to non-irradiated females. The animals received a total of 1000r ^{60}Co gamma rays delivered as two 500r exposures with a dose rate of 90r/min separated by 24 hours.

Sampling of progeny consists of obtaining blood by orbital puncture and one kidney by surgery. Subsequently, electrophoresis is carried out on the red cell lysate and kidney homogenate from each animal. It is anticipated that approximately 3000 offspring from each of the crosses (DBA x C57; C57 x DBA) will be analyzed for mutations at each of the nine loci showing electrophoretic polymorphism.

MAJOR FINDINGS: After radiation the sterile period for the DBA/2J males was four months. Progeny testing is in progress; 800 animals (7200 loci) have now been tested and, as yet, no electrophoretic variants have been detected.

Twenty-six males of the C57BL/6J strain were irradiated 11 months ago and only one has recovered fertility. Indeed, only two litters (9 offspring) have been obtained from that one male. Several of the other males were sacrificed at 10 months post-irradiation and cytological examination indicated that almost no mature sperm were present.

An experiment is now in progress to investigate the nature of the strain difference in radiation sensitivity. Both cytological and genetic criteria will be used to evaluate the level of cytotoxicity induced.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of chemicals may increase the genetic load of the human population which may possibly present a health hazard. There are two approaches that may be considered to avoid such an event. First, use animal test systems to detect chemicals which are mutagenic and prohibit or limit their use. Second, use mutant alleles at selected loci in the human population and monitor these for several years to determine whether there is an increase in the frequency of these alleles. The system under development will be pertinent to both of these approaches. It will survey a large number of different loci to minimize the cost of testing chemicals for potential mutagenicity and since many of the enzymes are also found in the human population, it will also provide a base-line for the study of the mutation rate of electrophoretic mobility mutations in the human population. Furthermore, since most of the loci code for specific enzymes, purification and biochemical analyses can be employed to determine the nature of the mutational event, i.e., base-pair substitution and/or addition/deletion in the DNA.

PROPOSED COURSE: Males of the C57BL/6J strain will be irradiated at a dose that will yield only a 3-5 month sterile period. The males will be mated to DBA/2J females and offspring will be tested for induced enzyme mutations.

A major effort will involve the development of electrophoresis protocols for the search of additional enzyme loci differences between DBA/2J and C57BL/6J strains.

Additional efforts will be directed toward the study of biochemical parameters of various enzymes to determine the feasibility of utilizing these in screening procedures.

Serial No: NIEHS-EM-011
1. Environmental Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Detection of New Protein Polymorphisms Using Polyacrylamide Electrophoresis"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Heinrich V. Malling, Ph.D.

OTHER INVESTIGATORS: Larry D. Claxton, Biologist and H. E. Walburg, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.1
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: This investigation attempts to detect new electrophoretic protein polymorphism in mouse blood serum. The inheritance of these proteins was also attempted using mouse strains with various genetic markers. Recent studies by other investigators have attempted to use specific serum proteins as a specific locus test for mutagenesis. The greater the number of loci to be examined, the more advantageous the test. This investigation will hopefully increase the number of loci.

METHODS EMPLOYED: The separation of blood serum proteins from 10 strains of inbred mice is accomplished using acrylamide electrophoresis. Upon finding an electrophoretic variant of any of the serum proteins, the appropriate genetic crosses are made. The serum of the offspring from these multiple crosses is then examined using acrylamide electrophoresis.

MAJOR FINDINGS: Five new pre-albumin bands have been found. The inheritance of one of the bands (Pre-albumin 1a) has been studied and has been found to be 2.25 map units from the brown locus.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings increase the number of loci that can be assayed for electrophoretically. This can, therefore, increase the number of loci used in an electrophoretic point mutation system. This locus can also be used to detect mutations in the closely linked brown locus which result from deletions.

PROPOSED COURSE: The inheritance of the other polymorphisms found in the pre-albumin bands will be studied. The role of the sex-determination of expression of these loci will be explored.

PUBLICATIONS

Claxton, Larry, Malling, H. V. and Walburg, H. E.: Serum pre-albumin in inbred strains of Mus musculus. Biochemical Genetic (in press).

Serial No: NIEHS-EM-012
1. Environmental Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Use of Mammalian Somatic Cells in the Detection of Mutational Events"

PREVIOUS SERIAL NUMBER: NIEHS-MB-008

PRINCIPAL INVESTIGATOR: Heinrich V. Malling, Ph.D.

OTHER INVESTIGATORS: Larry D. Claxton

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.1
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this investigation is to develop methods by which the frequency of somatic mutation at several genetic loci in experimental animals can be measured. Since blood is a readily available source of somatic cells that have a high turnover rate, we will initially attempt our studies with the use of blood. However, other tissues will be examined at a later date by a variety of techniques. Although we will attempt to explore human hemoglobinopathy method and a rabbit embryonic hemoglobin system, a thorough literature search will be attempted in order to find other possible systems. The detection of mammalian somatic mutation would provide advantages in mutation research for the following reasons: (1) human cell samples could possibly be used for the study of mutational rates in man, (2) immunologic reactions common to other systems could be avoided, (3) chronic studies of compounds in low doses could be initiated and (4) fewer animals and smaller facilities could be maintained for the study of mammalian mutagenesis.

METHODS EMPLOYED: The types of hemoglobin found in human hemoglobinopathies can be demonstrated by several methods: histological methods, physical methods such as lysis, and immulogical methods. We shall concentrate our efforts on the physical and immunological methods in attempt to detect and score mutant cells among a large number of normal cells. Since no usable hemoglobinopathy has been demonstrated in research animals, we will score for rabbit embryonic hemoglobin in adult animals. This hopefully will give us comparable systems in man and on research animals.

MAJOR FINDINGS: Although work in this area is in its infancy, we feel that early results demonstrate that a somatic mutation system in mammals is feasible. We know, for instance, that genetic variations in the presently tried systems do exist. It has also been demonstrated that one can locate the rare mutant cell in a population of normal cells with histological and immunologic techniques.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Simply stated, this new mutation system would allow the determination of increased mutational events within an individual animal. The system could be applied directly to human studies. Common disadvantages found in present mammalian system (such as, very large numbers of animals, immunological interference, lack of chronic studies) could be applied directly to human studies. Common disadvantages found in present mammalian systems (such as, very large numbers of animals, immunological interference, lack of chronic studies) could be overcome.

PROPOSED COURSE: Future efforts will try to determine the actual feasibility of these two systems and will also try to create other possible systems.

Serial No: NIEHS-EM-013
1. Environmental Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Use of Male Germinal Tissue in the Detection of Mutational Events"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Heinrich V. Malling, Ph.D.

OTHER INVESTIGATORS: Nivedita Mitra, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.1
Professional:	1.1
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this project is to develop methods for measuring the frequency of point mutations in male germinal tissue, for example mature sperm. Initial studies along these lines involve the use of histochemical staining techniques. The development of such a system would be advantageous because readily obtainable germ cell samples could be used for studying the mutation frequency in man. It would also provide an assay procedure to determine the effect of chronic levels of exposure because genetic damage will accumulate in the spermatogonial cells.

METHODS EMPLOYED: The enzymes sorbitol dehydrogenase and glucose-6-phosphate dehydrogenase which occur in sperm, can be readily detected by staining techniques. With the use of standard staining procedures variation in the co-enzyme, or by using different inhibitors specific to the enzyme it should be possible to detect individual variant cells.

MAJOR FINDINGS: Although the work at this stage is very preliminary, standardization of the staining technique should enable us to detect aberrant stained cells which are indicative of mutational events.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The present test system would permit the detection of mutational events within a single individual that could be used to monitor the human population as well as to evaluate the genetic consequences of accidental exposure to a mutagenic agent.

PROPOSED COURSE: The future plans include testing out the present system to determine the spontaneous frequency of variant cells as well as the frequencies induced by various mutagenic agents.

Serial No: NIEHS-EM-014
1. Environmental Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Development of a Mutation Detection System Based Upon New Drug Resistant Mutants in a Neurospora Heterokaryon"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: George R. Hoffmann, Ph.D.

OTHER INVESTIGATORS: Heinrich V. Malling, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: The major objective of this investigation is to develop a new system for the detection of forward mutations in a heterokaryon of Neurospora crassa. Since the method is based upon mutants which are resistant to the effects of 8-azaguanine, a secondary objective is to characterize the azaguanine-resistant strains.

METHODS EMPLOYED: Characterization of the mutants has involved both growth studies and linkage group determinations. By means of the linkage studies, the locus designation aza-3 was assigned to a Linkage Group III marker which confers azaguanine resistance in the strain used for mutation detection in the heterokaryon. A mutant at the aza-3 locus is recessive to its sensitive allele when incorporated into a heterokaryon. The heterokaryon, therefore, is sensitive to the effects of azaguanine. A method has been developed for the selection of azaguanine-resistant mutants in a heterokaryon carrying the aza-3 marker.

MAJOR FINDINGS: In addition to a description of aza-3 mutants, we have shown that in the proper selective medium, mutants resistant to azaguanine can be detected in the heterokaryon carrying the recessive aza-3 marker. An increased frequency of mutants was detected following treatment of conidia either with x-rays, ultraviolet light, ethylmethanesulfonate, or the acridine mustard ICR-170.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The new mutation detection system has been shown to have potential for development into a useful method for the detection of environmental mutagens. Being a forward mutation system, it is expected that a variety of genetic lesions can produce detectable mutants. Another feature of the new system which should permit the detection of a broad spectrum of genetic alterations is that the technique is being developed for use in a heterokaryon. By selecting for resistance in a heterokaryon carrying the resistance marker, one should detect a range of genetic alterations much like those which occur in diploid organisms. Complementation would permit survival of mutants bearing chromosome deletions or other alterations that would be lethal in homokaryotic strains. Developing the new system in a heterokaryon also provides for a specific locus system in which results can be interpreted in terms of mutations in one gene rather than at an unspecified number of loci conferring a given phenotype.

PROPOSED COURSE: Studies now in progress include a more refined genetic mapping of azaguanine-resistant mutants and the improvement of selection conditions for the detection of azaguanine-resistant mutants in the heterokaryon.

Serial No: NIEHS-EM-015
1. Environmental Mutagenesis Branch
2. Biochemical Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolism of Dialkylnitrosamines to Mutagens by Mouse Liver Microsomes"

PREVIOUS SERIAL NUMBER: NIEHS-MB-010

PRINCIPAL INVESTIGATOR: Christopher N. Frantz, M.D.

OTHER INVESTIGATORS: Heinrich V. Malling, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVES: Some chemicals may be harmless to an organism until that organism metabolizes the chemical to a reactive product. Dimethylnitrosamine (DMN) and diethylnitrosamine are such chemicals. When metabolized by mammals, mainly in the liver, they become toxic, carcinogenic, and mutagenic agents. Using DMN, an assay to detect mutagenic compounds has been developed by one of the authors (H.V. Malling) in another laboratory. This assay employs mouse liver to metabolize the chemical; bacteria is the organism in which mutations may be detected. It is our purpose to refine the above system in terms of variations in metabolic activity in the mouse livers, i.e., variations due to concentration, mouse strain differences, and treatment with enzyme inducers. Also, the biochemical pathway which metabolized DMN to a mutagen will be established.

METHODS EMPLOYED: The findings to date have been obtained using either supernatant of mouse liver homogenate or a purified microsomal fraction from mouse liver incubated with DMN in the presence of S. typhimurium G46, increasing the number of bacteria reverting from histidine dependence to histidine independence. Simultaneous assays of the microsomes for the production of formaldehyde during demethylation of DMN are performed. Demethylation is thought to be the reaction which metabolized DMN to a potent mutagen.

MAJOR FINDINGS: In refining the assay system, it was discovered that enzyme concentration is critical to the yield of mutations, and therefore to the potential usefulness and reliability of the assay. Above the critical enzyme

concentration mutation yield becomes linear in relation to amount of enzyme. Therefore, it can readily be compared to other biological events.

Studies in the scientific literature have described a correlation between incidence of liver tumors from DMN and level of liver DMN demethylating activity in rats. Two agents reported to decrease DMN demethylase activity in male rats, 3-methylcholanthrene and phenobarbital, increase the ability of the livers of male mice and rats to metabolize DMN to a mutagen. Secondly, a marked variation was found between strains of mice in ability to metabolize DMN to a mutagen. In rats and all strains of mice tested, with and without induction, there is a simple linear correlation between activation of DMN to a mutagen and DMN demethylase activity. DEN activation to a mutagenic product by mouse liver is also induced by phenobarbital and 3-methylcholanthrene, but variation between mouse strains does not follow the same pattern and DMN metabolism, thus indicating that different enzymes activate DMN and DEN.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has now been refined to a highly sensitive assay system which allows quantitative investigation of metabolic activation of potentially mutagenic compounds.

The discovery of differences between effects of inducers on mutagenicity and carcinogenicity calls into question the attractive hypothesis that activation of dialkylnitrosamines to alkylating agents is the rate controlling step in dialkylnitrosamine carcinogenesis, that more tumors will be induced where more short-lived active products are formed, and that activation of dialkylnitrosamines to toxic, carcinogenic, and mutagenic products are one and the same metabolic event.

PROPOSED COURSE: The range of mutations detectable with S. typhimurium hisG46 is limited. Also, bacteria are genetically much simpler than mammalian cells. Therefore, the microsomal mutagenicity assay will be adapted for use with a L5178Y mouse lymphoma cell culture forward mutation system developed by D. Clive, which detects a much wider range of genetic damage. The assay system will then be refined for quantitative investigation of a wide range of mutagens which require mammalian metabolic activation.

Serial No: NIEHS-EM-016
1. Environmental Mutagenesis Branch
2. Mammalian Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Synergistic Effects of Radiation and Chemical Mutagens on Spermatogonia in Mice"

PREVIOUS SERIAL NUMBER: NIEHS-MB-021

PRINCIPAL INVESTIGATOR: William Sheridan, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: Several investigations using known chemical mutagens have failed to show any indication of induction of dominant lethal mutations in the spermatogonial stem cells of mice. Studies with irradiation have shown that delivering a dose in two fractions increased the mutation rate, thus implying that the first fraction caused the stem cells to enter a more sensitive state by the time of the second fraction. It is our purpose to investigate whether pre-treatment with irradiation will sensitize spermatogonia to induction of mutations by chemical treatments following the irradiation.

METHODS EMPLOYED: Adult male mice were irradiated with 300 R of gamma rays. Twenty-four hours after irradiation they were given a dose of a known potent chemical mutagen (EMS) and thereafter mated in dominant lethal dissection studies. The animals went through a sterile period caused by the irradiation after which the germ cells tested corresponded to cells in the spermatogonial stem cell stage at the time of treatment. Results are being compared with those of animals receiving only irradiation or only chemical treatment as well as with those of a control group in order to determine any increase in the rate of dominant lethal mtuations. Samples of mice from all groups will be examined cytologically to determine the rate of induced chromosome aberrations.

MAJOR FINDINGS: In dominant lethal studies interpretation of rates of intra-uterine death have been complicated by apparent environmental factors effecting fertility of the test and control animals. Low pregnancy rates (30%-50%) and

excessively high intra-uterine death rates (> 20%) in controls tend to mask any increase in dominant lethal frequencies in the test group. Cytological preparations are currently being made on all groups and should be ready for analysis in the near future. Dependent on results obtained in the cytological study, decision will be made regarding the feasibility of repeating the dominant lethal dissection study.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mutations occurring in spermatogonial stem cells are of major concern since they not only will deliver large numbers of germ cells carrying identical mutations, but will do so for the remainder of the individuals' fertile life. Knowledge of the degree of sensitivity of stem cells to mutation induction is pre-requisite to studies intending to estimate the risks from mutagenic agents.

PROPOSED COURSE: Future studies will investigate the effects of presenting chemical mutagens in fraction doses to determine whether there will be a sensitizing effect comparable to pre-treatment with irradiation. A cooperative program with biochemists will be entered upon to determine the underlying mechanism of variation in sensitivity in stem cells.

Serial No: NIEHS-EM-017
1. Environmental Mutagenesis Branch
2. Mammalian Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Comparisons of the Effects of Chemical Mutagens on Somatic Cells and Germinal Cells in Mice"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Eugene R. Soares, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.5
Professional:	0.5
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: This project will be designed to explore the feasibility of using somatic cells (i.e., lymphocytes) to screen for chemically induced chromosomal abnormalities. Inclusive in this project is the study of the relative sensitivities to chemical mutagens of germ cells (spermatogonia, spermatocytes, etc.) and lymphocytes.

METHODS EMPLOYED: Adult male and female mice will be exposed to a known chemical mutagen (TEM). Subsequent to exposure blood will be withdrawn from these animals, cultured, and the lymphocytes prepared for cytological examination and karyotyping. Also, following treatment males will be mated according to the procedure used in the dominant lethal assay. Treated females will be mated and following conception, zygotes will be examined for the presence of chromosome abnormalities of micronuclei. Testicular sections from treated males will also be examined for the presence of induced abnormalities. The sensitivities of somatic and germinal cells will be compared.

MAJOR FINDINGS: This project was initiated in March 1974.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A major drawback in using mammals for screening for chemical mutagens is the relatively long generation time as compared to lower organisms. The use of somatic cells for screening procedures would eliminate this problem and would allow rapid and reliable screening of large numbers of animals. This project will provide

information concerning the relative sensitivities of these cells and may enable extrapolation from somatic cells to germinal cells. Similarly, such information could easily be adopted to use among human populations.

PROPOSED COURSE: A variety of chemical agents will be tested in several strains and hybrid mice. The effects of acute and chronic administration of the compounds will be considered.

Serial No: NIEHS-EM-018
1. Environmental Mutagenesis Branch
2. Mammalian Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Studies of the Effects of Route of Administration on the Sensitivity of Different Mouse Strains to Mutagenic Agents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Eugene R. Soares, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: Earlier studies designed to test the toxicological sensitivity of various compounds have shown marked differences in the sensitivity between the various strains of mice used. Our present studies use known chemical mutagens and are designed to confirm the above finding as well as determine variability of mutagenic sensitivity between routes of administration and between and within strains of mice.

METHODS EMPLOYED: Mice of two inbred strains and one hybrid were given three different doses of EMS (an alkylating agent and potent mutagen). The doses were administered either intraperitoneally (I.P.) or by gavage. All mice were observed for the toxic effects of this chemical and subsequently mated according to the standard scheme employed in the dominant lethal assay.

MAJOR FINDINGS: This project was started in November 1973 and data collection has not yet been completed. Preliminary data available at this time indicate the existence of differences in toxicity but not mutagenicity of EMS between strains and between route of administration. Complete analysis of these data is not yet possible.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The importance of genetic and route of administration of chemical mutagens and the background relationship with mutagenic and toxicological sensitivity has not been studied. Test systems as they are currently designed are restricted to

the use of one or a few inbred strains and, with few exceptions, consider only the effects of i.p. injections of the test compounds. Since human populations are genetically heterogeneous and since exposure of humans to various compounds, either toxic or mutagenic is by routes other than i.p., it is extremely important that the varying effects of genetic background and route of administration be tested. Such effects must be studied before reliable screening procedures can be developed which would allow extrapolation to human populations or estimates of risk.

PROPOSED COURSE: Future efforts will be directed toward elucidating the varying effects of a series of different compounds on a number of strains. Efforts will also be made to determine the genetics of the different patterns of observed sensitivities.

Serial No: NIEHS-EM-019
1. Environmental Mutagenesis Branch
2. Mammalian Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Comparative Sensitivities of Different Mouse Strains to Mutagenic Agents"

PREVIOUS SERIAL NUMBER: NIEHS-MB-020

PRINCIPAL INVESTIGATOR: William Sheridan, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	3.0
Professional:	1.0
Other:	2.0

PROJECT DESCRIPTION

OBJECTIVES: Earlier studies, utilizing irradiation as the mutagenic source, have shown that the genome of the test organism is a major factor in the determination of its susceptibility to genetic damage. It is our purpose to investigate to what extent such genetically caused differences in sensitivity pertain to other mutagenic agents. We also want to know if the relative level of sensitivity of a defined genome to induction of mutations is constant for all agents or variable depending on the mutagen used.

METHODS EMPLOYED: We want to accrue a battery of test strains, each showing a distinct level of sensitivity to mutation-induction, covering a range from highly sensitive to highly resistant. These strains will be selected on the basis of induction of dominant lethal mutations by a known potent mutagen (EMS). They will then be challenged by a successive array of other known or suspected mutagenic agents to discover any changes in their relative levels of sensitivity or indications that suspect compounds may be mutagenic.

MAJOR FINDINGS: Preliminary screening of five inbred strains has been made. Apparent environmental factors affecting fertility of test and control animals have complicated assessment of the true sensitivities of the strains. There are indications of differences between some of the strains tested despite spuriously high intrauterine death rates in controls. These differences give promise of the success of the project, however, retests of these strains plus additional strains will be necessary before final choices of strains to be included in the test battery are made.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the gene pools of human populations by and large are extremely heterogeneous, one would expect a broad spectrum of reaction to a mutagenic agent in the environment. Restriction of testing to single, often inbred, strains of another mammal such as the mouse will not give indications on a wide scale of what might be the effects of such an agent on the human population. By determining the effects of a compound on a battery of strains presenting a variety of genotypes and differing sensitivities, we will better be able to approximate what might be expected in a heterogeneous mammalian population such as man.

PROPOSED COURSE: Future efforts will be devoted to understanding the underlying genetic causes of differences in sensitivity to mutation induction, and the consequences of combining the genetic materials of strains with divergent sensitivities.

Serial No: NIEHS-EM-020
1. Environmental Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Safety Testing and Analysis of Environmental Compounds for Mutagenic Effect"

PREVIOUS SERIAL NUMBER: NIEHS-MB-014

PRINCIPAL INVESTIGATOR: Donald Clive, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.6
Professional:	0.3
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: We have developed an in vitro mutagen assay system utilizing thymidine kinase heterozygote ($TK^{+/-}$) L5178Y mouse lymphoma cells, which respond quite sensitively to treatment with known mutagens. Two classes of chemical mutagens (alkylating agents and hycanthone analogs) are currently being assayed in this system for the purpose of determining structure-activity relationships. The two classes of compounds represent mutagens of two distinct mechanisms of action (i.e., covalent alkylations of the DNA resulting primarily in base-pair alterations; and non-covalent intercalation into the DNA leading to spatial distortions of the base-pair sequences and ultimate frame-shift mutations.).

METHODS EMPLOYED: These are described in detail under Project No. NIEHS-M-004. Briefly, the mutagen assay involves exposing a culture of $TK^{+/-}$ L5178Y mouse lymphoma cells to the test compound, washing the cells, and allowing them to express any genetic lesions that might have been introduced. An identical culture is sham treated as a negative control. Following expression time the cells are plated in soft agar cloning medium supplemented with 5-bromo-deoxyuridine (BUdR); subsequent incubation kills off all but the $TK^{-/-}$ mutant cells which form from visible colonies after 7-10 days. Viable counts, reconstruction using stock $TK^{-/-}$ mutants and positive (EMS or hycanthone) controls permit us to evaluate quantitatively the extent to which the given treatment has enhanced the mutation rate of these cells.

MAJOR FINDINGS: Fourteen hycanthone analogs have been compared for dose-response curves. Correlations between chemical structure and (a) maximum observed mutagenicity, (b) cellular toxicity and (c) the shape of dose-response curves have been determined. An additional anti-schistosomal drug, SQ 18,506, has also been examined and found to compare with hycanthone in its mutagenicity. The alkylating agents ethyl methanesulfonate, methyl methanesulfonate and ethylene dibromide have been studied both for dose-response curves and for mutagenic efficiencies (see Project No. NIEHS-EM-023).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Essential to the early detection and control of environmental mutagens is a safety testing program utilizing a simple, sensitive mutational assay system, such as the one used here (see also Project Nos. NIEHS-M-004 and NIEHS-M-005). The demonstration of the mutagenicity of hycanthone in mammalian cells has given additional impetus to safety testing at NIEHS. The studies on structure-mutagenic activity relationships are aimed at aiding in the development of a safe efficacious antischistosomal drug, and in better understanding the molecular mechanism(s) by which these drugs exert their mutagenic effects. Both the alkylating agents and the hycanthone analogs are being studied to better evaluate the quantitative relationships existing between induced damage to DNA and resultant mutant frequency.

PROPOSED COURSE: The mutagenicity of AF-2 - a food additive widely used in Japan and mutagenic in non-mammalian cells -, of dichlorous (active ingredient in NO-PEST strips) and of other suspected mutagens of environmental significance will be examined.

PUBLICATIONS

Clive, D., Flamm, W. G., and Machesko, M. R.: Mutagenicity of hycanthone in mammalian cells. Mutation Research 14: 262-264, 1972.

Clive, D., Flamm, W. G., Machesko, M. R., and Bernheim, N. J.: A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Research 16: 77-87, 1972.

Clive, D.: Recent developments with the L5178Y TK heterozygote mutagen assay system. Environmental Health Perspectives 6: 119-126, 1973.

Serial No: NIEHS-EM-021
1. Environmental Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Mutational Assay System Utilizing Mammalian Cells"

PREVIOUS SERIAL NUMBER: NIEHS-MB-013

PRINCIPAL INVESTIGATOR: Donald Clive, Ph.D.

OTHER INVESTIGATORS: Jo-Anna Spector

COOPERATING UNITS: None

MAN YEARS

Total:	1.2
Professional:	0.6
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: Because of their large numbers, relatively short generation times, ease of handling and biological relevance to mammalian genetics, mammalian cells grown in suspension culture offer a highly sensitive system for assaying the mutagenicity of environmental agents. In conjunction with biochemical studies (see Project No. NIEHS-MB-012,-017 and -018) it is expected that the specific locus mutational assay system which we have developed can be used successfully in elucidating the mechanism by which a variety of such mutagens exert their effects in mammalian cells. This project is aimed at evaluating and extending the utility of the mouse lymphoma mutagen assay system, especially in the area of accurate quantitation of genetic damage in relation to exposure and chemical structure.

METHODS EMPLOYED: Thymidine kinase- (TK-) deficient mutants of L5178Y mouse lymphoma cells are isolated as resistant colonies growing in soft agar cloning medium supplemented with 50 µg/ml of 5-bromodeoxyuridine (BUdR); this medium selects against TK-competent cells by virtue of their ability to affect a TK-mediated phosphorylation of BUdR which is ultimately lethal. TK-deficient cells are selected against by a medium supplemented with thymidine (T), hypoxanthine (H), methotrexate (M, to inhibit folic acid metabolism) and glycine (G) (THMG medium), whereas TK-competent cells can utilize the exogenous thymidine to overcome the methotrexate block and, hence, survive. Spontaneous mutation rates (forward and reverse) are determined by the rate of mutant accumulation in a culture and, where practicable, by a fluctuation test. TK mutant phenotypes are confirmed by determining the rate at which their cell

lysates can phosphorylate thymidine (TK assay). The efficiency of mutant recovery in each of the selection media is determined in reconstruction experiments, utilizing standard mutant stocks. Potential chemical mutagens are assayed as follows: test cells of known genotype are treated with the compound for 2 h, the cells are washed, allowed to express genetic lesions for 48-72 h, cloned in selective cloning medium and scored for surviving mutant colony frequency after 10-11 days incubation.

Development of a protocol for simultaneous assessment of chromosomal alterations in the same populations of cells which are being processed for specific gene mutations is also in progress using standard cytogenetic procedures for the literature.

MAJOR FINDINGS: In the course of examining several derivatives of hycanthone for structure-mutagenicity relationships in the TK-heterozygote L5178Y mouse lymphoma system, marked deviations from traditional dose-response curves have been noted. Over part of the concentration range examined, a number of these compounds show significantly decreasing mutagenicity with increasing concentration (2 h exposure). This contra-mutagenic effect is most significant for the indazole derivative of lucanthone, IA-5, and is also prominent for lucanthone itself. At 30 μM, IA-5 exerts maximum mutagenicity (40 mutants/10^4 surviving cells) and toxicity (0.08% survival relative to untreated controls). At concentrations between 70 and 85 μM the mutagenic and toxic effects are markedly reduced (6-fold reduction in mutagenicity, 60-fold increase in survival, relative to low dose treatment). Growth curves for cultures treated at various concentrations support a mutagenic origin of the toxicity observed at 30 μM, thereby ruling out the possibility that the thymidine kinase (TK) locus used in these studies behaves atypically. Moreover, at 85 μM these same curves indicate a near absence of genetic damage beyond an early killing of cells during treatment, again in agreement with the specific locus mutational data.

Preincubation studies with IA-5 have shown no indication of any alteration to the molecule at 85 μM in culture medium with or without cells. In synchronized cells both mutagenicity and survival are maximal in S phase at 30 μM (but not at 85 μM). This anomalous correlation between lethal mutations (minimum frequency in S) and mutations at the TK locus (maximum frequency in S) is currently under investigation. In order to determine more specifically the requirement of this compound for DNA synthesis in the expression of its mutagen properties, the incorporation of [^{3}H]-thymidine into DNA was studied. A good correlation between extent of DNA synthesis as measured by thymidine incorporation and mutagenicity was found (DNA synthesis reduced six-fold at 85 μM relative to 30 μM, mutagenicity also reduced six-fold; a similar two-fold reduction in each of these parameters was found using lucanthone at high and low concentrations).

The capacity for one of these analogs of hycanthone to exert a contra-mutagenic effect at high concentrations appears to depend on certain specific molecular features, notably a planar (indazole) structure, a methyl group in the 4-position and the presence of methyl or ethyl groups towards the distal end of the side-chain.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is integrated with the development of a mouse-mouse host-mediated assay for chemical mutagens (Project No. NIEHS-M-005) and with studies of the mutagenicity of environmental agents (Project No. NIEHS-M-006) and shares with their significance. In conjunction, the three projects aim to (1) screen environmental compounds for mutagenicity; (2) distinguish between mutagenicity of a parent compound and of its metabolites; and (3) characterize mechanisms by which such mutagenic effects are produced or, in certain instances, ameliorated. This ability to diminish both the toxic and mutagenic effects of such compounds as lucanthone and IA-5; however, impractical current efforts might appear, hold the promise of a pathway to the oft-sought anti-mutagen. More practically, these data are important for the continued appraisal of this mutagen assay system, for its strengths and weaknesses, and for a comparison with other such systems.

PROPOSED COURSE: Intensive analysis of the roles of DNA synthesis and repair in the described contra-mutagenicity phenomenon and of the molecular features of certain mutagens which can exert such a contra-mutagenic effect will be pursued. A quest for non-mutagenic compounds which can mimic this effect by co-therapy with potent mutagen concentrations will be initiated. The potential for assaying chromosomal aberrations together with specific locus mutations is being developed.

PUBLICATIONS

Clive, D., Flamm, W. G., Machesko, M. R., and Bernheim, N. J.: A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Research 16: 77-86, 1972.

Clive, D., Flamm, W. G., and Patterson, J. B.: Specific locus mutational assay systems for mouse lymphoma cells. In Hollaender, A. (ed.): Chemical Mutagens: Principles and Methods for Their Detection. Plenum Press, N.Y., 1973, pp. 79-103.

Clive, D.: Recent developments with the L5178Y TK-heterozygote mutagen assay system. Environmental Health Perspectives 6: 119-126, 1973.

Clive, D.: Mutagenicity of thioxanthenes (hycanthone, lucanthone and four indazole derivatives) at the TK locus in cultured mammalian cells. Mutation Research (in press).

Serial No: NIEHS-EM-022
1. Environmental Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Investigation of Thymidine Kinase Enzymes in Independently Derived Mutant Lines of L5178Y Mouse Lymphoma Cells"

PREVIOUS SERIAL NUMBER: NIEHS-MB-012

PRINCIPAL INVESTIGATOR: Donald Clive, Ph.D.

OTHER INVESTIGATORS: Peter Voytek, Ph.D., J-A. Spector

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: A number of genetic test systems have been developed, utilizing a spectrum of organisms ranging from prokaryotes to whole mammals. None is perfect for assessing mutagenic hazards to man: those organisms which best combine sensitivity and analytical capability are phylogenetically most distant from man, while, conversely, those species closest to man combine the disadvantages of long generation times, large size and great expense to their use. A compromise between those two extremes is afforded us by the use of cultured mammalian cells, which combine large numbers and relatively short generation times, ease of handling, low cost, a mammalian genome, and other properties characteristic of mammalian cells. However, such systems still lack the level of biochemical and genetic comprehension which elevates a mutagen assay system to a powerful genetic tool. Such understanding must develop as a wedding of genetic and biochemical studies on a number of mutant lines.

Genetically, the thymidine kinase (TK) locus of L5178Y mouse lymphoma cells can be mutated by a variety of physical and chemical agents. TK-deficient mutants can be isolated and later reverted to yield TK-competent mutants. (See Project Nos. NIEHS-M-004 and NIEHS-M-006.) Thymidine kinase activities on the cell lysates confirm these designations. What is not known - - and what this project hopes to elucidate - - is whether a control gene is being mutated to shut off production of thymidine kinase, or whether the thymidine kinase structural gene itself is being affected. In the first case, analyses of all TK-competent lines should show no biochemical variations among the various

thymidine kinase enzyme sources. On the other hand, the combination of a forward and a reverse mutation in the structural gene need not yield precisely the same enzyme in the various revertant lines. And, even though enzyme activities are similar among these revertant lines (an expected result, because the very existence of the mutant is predicated on its possessing good thymidine kinase activity) other characteristics of the enzyme might differ widely (e.g., electrophoretic mobility, alterations in allosteric responses to activators and inhibitors, kinetic parameters).

METHODS EMPLOYED: Forward mutation to $TK^{-/-}$ from $TK^{+/+}$ culture were induced by ethyl methanesulfonate, while $TK^{+/-}$ revertants were allowed to accumulate spontaneously. Attempts to analyze thymidine kinase enzymes by gel electrophoresis were foiled by the insolubility of the wild-type ($TK^{+/+}$) enzymes. Instead, enzyme solubility in saline of LiCl, ATP substrate requirements, and enzyme kinetics were compared for the various heterozygotes and the initial homozygote. Variations between a heterozygote and the $TK^{+/+}$ in any of these parameters was considered as evidence for a structural gene mutation.

MAJOR FINDINGS: Alterations in all three criteria - enzyme solubility, substrate dependence and enzyme kinetics - were observed among the various revertant clonal lines examined compared with the wild-type enzymes. The conclusion that structural gene mutations are being detected at the TK locus, as opposed to a regulatory gene mutation, is supported by these data, at least for the mutagen EMS.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Complete understanding of how a particular mutagen acts depends ultimately on the level of sophistication of the genetic test system being used. This project aims at increasing our knowledge of the already proven TK heterozygote L5178Y mouse lymphoma mutagen assay system.

PROPOSED COURSE: Characterization of the thymidine kinase enzymes derived from various $TK^{+/-}$ mutants will be continued using different classes of mutagens.

Serial No: NIEHS-EM-023
1. Environmental Mutagenesis Branch
2. Somatic Cell Genetics Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Dosimetry of Chemical Mutagens"

PREVIOUS SERIAL NUMBER: NIEHS-MB-018

PRINCIPAL INVESTIGATOR: J. B. Patterson, Ph.D.

OTHER INVESTIGATORS: Donald Clive, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	0.2
Professional:	0.2
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Before we can deal fruitfully with the important question of how mutations are mediated in mammalian cells by chemical mutagens, we must first attempt to answer another question. That is -- what is the quantitative relationship between the number of lesions introduced into genetic material and the number of mutations which result from exposure to that agent? We are attempting to answer this two-part question in two distinct operations. We first have to obtain data on a variety of known chemical mutagens in order to compare the relative mutagenic efficiencies (RME) of the compounds. We also must determine to what extent these compounds have an affinity for and a tendency to react with DNA. We wish to ultimately determine, for example, why only one EMS-induced alkylation out of about 10,000 results in an observable mutation in L5178Y mouse lymphoma cells.

METHODS EMPLOYED: Our operations are divided into two specific areas, (1) mutagen testing in mammalian cells and (2) measurement of the association of mutagens with DNA on a molecule per nucleotide basis. The mutagen testing methods are described in detail in NIEHS-M-004. The extent of DNA-mutagen interaction is measured by the following methods: (1) direction by the exposure of cells to radioactive alkylating agents, (2) by special perturbation during the interaction of DNA with various planar, heterocyclic compounds (e.g., hycanthone, and its analogs), and (3) the kinetics of depurination of DNA following interaction as described in NIEHS-M-002.

MAJOR FINDINGS: Induced mutant frequencies and number of alkylations per gene have been determined as a function of concentration of ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS) and ethylene dibromide (EDB). From these data it has been possible to calculate the number of mutations induced per alkylation - i.e., the mutagenic efficiencies - for each of these agents. For EMS the mutagenic efficiency stays approximately constant, ranging between 0.5 - 1.5 mutations/10^4 alkylations, up to 10 mM concentration; at higher concentrations the mutagenic efficiency increases linearly and rapidly with concentration to a measured high of 10 mutations/10^4 alkylations. MMS is effective at much lower concentrations than is EMS, resulting in technical difficulties in assaying alkylation levels at the lower concentrations. Over the range studied the mutagenic efficiency of MMS increases linearly and 5 times as rapidly with concentration as does that of EMS. Maximum mutagenic efficiency for MMS occurs at 0.75 mM and is 20 mutations/10^4 alkylations. Preliminary data on EDB yield mutagenic efficiencies between 1-4 mutations per 10^4 alkylations over the narrow concentration range studied.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In assessing the potential health hazard a given mutagen might present to man, a number of extrapolations must be made from the test systems. For our test system, these include: somatic vs. germinal cells; single cells vs. whole animals, and mouse cells vs. human cells. All of these factors represent potentials for ameliorating or enhancing the mutagenic effects of a given agent, especially by altering the extent of interaction between mutagen and DNA. By quantitating this interaction for various test systems, the confidence with which mutagenic data can be meaningfully extrapolated to man is greatly improved.

PROPOSED COURSE: If techniques can be developed, we plan to examine other classes of chemical mutagens (e.g., hycanthone and its analogs) which do not bind covalently to DNA.

Serial No: NIEHS-EM-024
1. Environmental Mutagenesis Branch
2. Population Genetics & Epidemiology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Genetic Variability in Sensitivity of Lymphocytes to Physical Mutagens"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Charles H. Langley, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: Considerable indirect evidence suggests the existence of significant genetic variability in the sensitivity of individuals to toxic environmental agents. Pharmacogenetic investigations have revealed several genetic polymorphisms for enzymes involved in the metabolism of widely used drugs. Xeroderma pigmentosa is a well-studied genetic disease characterized by high sensitivity to ultraviolet light, the occurrence of multiple skin cancers and DNA repair deficiency. Although this is a rare and severe genetic variation, it serves as an indication of source and importance of genetic variability in environmental sensitivity. In order to study the role of genetic variability in the etiology of certain environmental health problems, we have begun to develop an assay of sensitivity. To apply such an assay on the scale necessary to examine genetic variability it must have certain properties: (1) it must be performed on readily available tissues; (2) it must be in vitro to allow utilization of known mutagens and carcinogens; (3) it must be efficient to allow extensive population sampling; and (4) it must be reproducible.

METHODS EMPLOYED: The technique we are developing at the present consists of the measurement of the killing kinetics of purified lymphocytes. In the initial phase x-rays and ultraviolet light will be used because of the ease of dosimetry and to avoid some of the possible effects of metabolism. The first effort will be to relate the sensitivity of whole body exposure to x-ray (already well-characterized) to that of the lymphocytes in various inbred strains of mice.

MAJOR FINDINGS: The project was initiated in January 1974.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evaluation of risk of environmental hazards should take human variability into account. At the present there is little or no data upon which the magnitude of this variability can even be estimated. It is important that tools be available that allow us to predict the frequency of individuals who may be as much as 10 and 100 times more sensitive to environmental mutagens and carcinogens.

PROPOSED COURSE: The future of this line of research depends on the progress in the development of *in vitro* assays for mutant cells in humans. If, in several years, we can efficiently measure genetic variability in the somatic mutagenic sensitivity, then such a technique is, of course, the preferred and more relevant assay. However, at the present we have no such assay available. The proposed cell-killing assay is workable at the present and should reflect with adequate efficiency the cogent biological parameters.

Serial No: NIEHS-EM-025
1. Environmental Mutagenesis Branch
2. Population Genetics & Epidemiology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Genetic Variability and Its Relationship to Environmental Variation"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Charles H. Langley, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS

Total:	1.0
Professional:	0.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: Recent studies have implicated environmental polymorphism as an important parameter in the interpretation of existing genetic polymorphism, especially allozymic polymorphism. We are attacking the question from two angles: (1) theoretical modeling of evolution dynamics in the presence of environmental variation and (2) experimental investigations of the relative sensitivity of various genotypes to environmental hazards. In cooperation with statisticians in the Environmental Biometry Branch and geneticists at North Carolina State University, we are analyzing gene frequency changes in response to insults of environmental chemicals. This will give some indication of the responsiveness of gene frequencies to environmental contamination.

METHODS EMPLOYED: In the theoretical analyses, Monte Carlo computer simultations are being run to investigate the relationships of environmental variability to genetic variability. In experimental work, laboratory populations of Drosophila are presently being utilized. These are killed off to a large extent (90%) with environmental agents and the gene frequencies at allozyme loci analyzed.

MAJOR FINDINGS: The project is just beginning to produce data. No results are available as yet.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent years it has become apparent that there is an enormous amount of genetic varia-

bility present in natural populations, including humans. The adaptive significance of this variation must be understood in order that we can judge the implication of new environmental changes and the genetic consequences of mutagenic environmental agents.

PROPOSED COURSE: If gene frequency changes prove to be indicators of environmental insult, we plan to investigate natural populations for evidence of response to known environmental contaminations.

Serial No: NIEHS-EM-026
1. Environmental Mutagenesis Branch
2. Population Genetics & Epidemiology Section
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Pigmented Retinal Epithelial Cells as an Assay System for Somatic Mutation"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: Galen R. Hasler, M.D.

OTHER INVESTIGATORS: Charles H. Langley, Ph.D.
Larry Claxton, Biologist

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0

PROJECT DESCRIPTION

OBJECTIVES: The investigation of the feasibility of a somatic mutation assay system with mouse pigmented retinal epithelial cells is being pursued. It is hoped that such an assay might provide a simple and sensitive screen for somatic genetic activity (mutagenesis and somatic crossing over) in mice.

METHODS EMPLOYED: A pilot project is being carried out using two inbred mouse strains which are crossed to form a hybrid which is heterozygous for albinism. By exposing these hybrids to x-ray or mutagens in utero, albino cells in the pigmented retinal epithelium may be produced either by somatic mutation or crossing over. Quantification of pigment changes would correlate to somatic mutagenesis and/or crossing over. Direct observation of the retina in vivo and histological techniques will be used. Positive control mice are available in the translocation stocks in which albinism segregates somatically producing patched patterns in the pigmented retinal epithelial.

MAJOR FINDINGS: Project is in initial stage.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many environmental agents are suspected of producing somatic mutations and crossing over in humans. At present there is no mammalian screening system available which can identify genetically active compounds in vivo. This preliminary research could lead to such an assay system.

PROPOSED COURSE: Research efforts will be to determine the actual feasibility of this assay system, based on preliminary results.

ENVIRONMENTAL TOXICOLOGY BRANCH

ENVIRONMENTAL TOXICOLOGY BRANCH
Summary Statement

The scientific efforts of this Branch are directed generally towards determining the mechanisms of toxicity of environmental agents. All aspects of acute, subchronic, and chronic toxicity are considered with special emphasis on reproduction and development. A better understanding of underlying mechanisms will hopefully lead to the development of more rapid, reliable, and inexpensive test methods than those currently available. Attempts are made to support strong independent basic research programs which are interrelated and directed towards specific goals.

The Environmental Toxicology Branch currently conducts research in three informal major organizational sections: (1) Fertility, (2) Reproduction and Ontogenesis, and (3) Biochemical Toxicology. Within this organizational structure, seven "workgroups" are defined and represent, in some cases, projects which are being de-emphasized to provide resources for research efforts which have greater priority and relevance to this Institute. The "workgroups" are (1) Fertility, (2) Reproduction and Ontogenesis, (3) Biochemical Toxicology, (4) Mammalian Tissue Culture, (5) Heavy Metal Toxicity, (6) Membrane ATPase, and (7) Marsupial Program. A brief summary of progress made by each workgroup follows:

Fertility. Doctors Lee, McLachlan, and Dixon have continued their studies of the effects of prenatal and/or postnatal exposure to environmental chemicals on fertility. It is apparent that rather low doses of environmental chemicals, including drugs, can dramatically alter the reproductive capacity of both male and female experimental animals.

Doctor Lee and coworkers have investigated unscheduled DNA synthesis in mouse spermatogenic cells using the velocity sedimentation cell separation technique and in vivo serial mating. Other studies attempt to better understand the synthesis and structure of messenger RNA in rat spermatogenic cells, define enzyme development and identify useful "marker enzymes" during spermatogenesis, and better describe the nature of the blood-testis barrier. Both in vitro and in vivo exposure of spermatogenic cells to known mutagens, e.g., MMS, followed by cell separation demonstrated that thymidine was incorporated into the acid insoluble fraction of premeiotic and spermatogonial cells. This interesting observation was dose and time dependent and confirmed the fact that post-meiotic cells (spermatids and sperm) are not capable of DNA repair. Alterations of selected testicular enzymes ("marker enzymes") correlated well with histologic changes and cell population alterations in seminiferous tubule cells following treatment of mice with procarbazine. Rather elegant studies of the transfer of chemicals from blood to rete-testes fluid (testicular fluid) in the rat suggest that the blood-testis barrier is very similar to that which protects the brain, the blood-brain barrier.

Doctor McLachlan and coworkers' experiments demonstrate that prenatal exposure to diethylstilbesterol (DES) adversely affects the reproductive capacity of the female offspring. Timed pregnant mice were treated with DES during the period of embryonic ovarian development (days 10 through 16 of gestation) in doses

ranging from 0.01 to 100 μg/kg. When the female offspring of these mice reached sexual maturity, they exhibited varying degrees of subfertility ranging from a significant reduction in total reproductive capacity to sterility. No differences were observed between control and DES-exposed offspring with regard to their estrous cycles or in the distribution of follicular types in their ovaries. The mechanism of this effect is being sought at the biochemical and physiologic level using reciprocal transplantation studies in which fertilized ova from DES-treated females are transferred to the uterus of control females and the corresponding control conceptuses are transplanted into the uterus of DES-treated offspring. These tests will evaluate the relative contributions of the oocyte and uterus to the observed reproductive failure. In vitro experiments were recently begun to isolate, explant, and culture ovarian primordial tissue from fetal mice in chemically defined media. This system, being developed in collaboration with Doctor Spalding, should provide information on the basic processes of oogenesis and folliculogenesis; and may provide a screening technique to determine the ovotoxic potential of environmental chemicals.

Reproduction and Ontogenesis. This group is primarily concerned with the development of better test methods for assessing the teratogenic potential of environmental agents and components that will have greater predictive value for man than current tests. A variety of in vitro and in vivo techniques are being used to define the reactive maternal and fetal factors which contribute to birth defects.

Doctor Staples and coworkers have determined that provera (medroxy progesterone acetate), a suspected teratogen in humans, produces cleft palates and other malformations in offspring of treated rabbits but is without effect in rats, and mice at comparable doses. Thyroparathyroidectomy of female rats at various ages prior to breeding was without teratogenic effect and thyroidectomy failed to protentiate the toxicity of dichlorvos. Mice were housed in sound-proof chambers throughout gestation and the incidence of spontaneous terata was compared to animals maintained in the Institute's animal quarters. No difference in the incidence of malformations was noted. Captan failed to demonstrate teratologic alterations following gestational treatment (days 6-15) or intrauterine administration of relatively large test doses. Tenuazonic acid, a mycotoxin derived from Alternaria, was demonstrated to be teratogenic in mice.

Embryos have been recovered from donor animals and cultured in vitro for varying periods, exposed to selected environmental chemicals, and then transferred to recipient females or examined for morphologic and/or chemical changes. Dimethyl sulfoxide (DMSO) and its metabolite, dimethyl sulfone, did not produce teratogenic changes following exposure of the blastocyst in culture and transfer to recipient animals. Continuous exposure (22 hours) of developing mouse embryos to microwave radiation which did not produce an increase in heat was without teratogenic effects. Tenuazonic acid failed to demonstrate teratogenic effects following addition of the chemical to in vitro cultures of mouse morula at the highest tolerated concentration.

Doctor Kimmel is investigating the teratogenic potential and toxic mechanism of various chelating agents and is studying their interaction with essential heavy metals. Doctor Kimmel has been at the NIEHS for less than a year and this project is only in its initial phases.

Doctor Maurer is attempting to better understand the biologic significance of uterine luminal secretions present during early pregnancy and to determine whether quantitative or qualitative changes in luminal secretions occur when animals are exposed to environmental factors. Significant differences in luminal proteins were detected in 4 to 6-day pregnant rabbits. Injection of progesterone prior to ovulation produces a premature appearance of uteroglobin in the luminal proteins which alters the uterine-embryo synchrony. Doctor Maurer has also achieved low temperature storage of mammalian embryos. Mouse and rabbit embryos has been frozen at -196°C for prolonged periods of time and thawed successfully as judged by subsequent *in vitro* and *in vivo* development. Freezing was without apparent teratogenic effect.

Doctor Moustafa has used micrurgical techniques for the direct administration of test agents into cultured mammalian blastocysts as another means of directly testing chemicals for their teratogenic effects. The micrurgical techniques has also been used to produce chimaeric animals. To date, thalidomide has been demonstrated to be embryolethal when tested by this method, but not teratogenic. Embryonic single-cells transplanted into rabbit blastocysts produced chimaeric offspring. Phenotypic differences in the eye, skin, and coat color indicated the successful participation of transplanted cells in host organogenesis.

Biochemical Toxicology. Scientists in this workgroup conduct independent research projects and provide basic biochemical support for the Branch.

Doctor Lucier continues to conduct an active high quality laboratory program while supporting the Branch and Institute in a variety of other ways. Doctor Lucier and coworkers are characterizing hepatic and extrahepatic UDP-glucuronyltransferase and N-acetyltransferase. The perinatal development pattern of these conjugative enzymes in different tissues is also being studied. Guinea pig p-nitrophenol (PNP) glucuronyltransferase was detected 25 days prior to birth while liver testosterone glucuronyltransferase activity did not appear until just before birth. These data along with kinetic studies suggest that there are at least two microsomal UDP-glucuronyltransferases. N-acetyltransferase, a supernatant enzyme, had equivalent fetal, newborn, and adult enzyme activities which were comparable at all periods tested. A single oral dose (5 μg/kg) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased liver cytochrome P-450, cytochrome b_5, biphenyl hydroxylation, benzpyrene hydroxylation, and PNP glucuronidation, but had no effect on testosterone glucuronidation or oxidative demethylation. Effects on hydroxylation and glucuronidation rates were observed at TCDD doses as low as 0.2 μg/kg and induction was still evident two months after treatment. Exposure of pregnant rats to TCDD increased liver glucuronyltransferase and benzpyrene hydroxylase activities 3-10 fold in newborn animals.

Doctor Woods and coworkers have continued their studies of the factors affecting hepatic heme synthesis during mammalian development. This work indicates that heme may facilitate the development of mitochondrial ALA synthetase during fetal development. ALA synthetase is the first and rate-limiting enzyme in hepatic heme biosynthesis in adults. These results suggest that the fetal enzyme is not rate-limiting until it becomes susceptible to repression by heme, a phenomenon which does not develop until near birth. ALA synthetase from

fetal rat liver mitochondria has been solubilized and purified approximately 30 fold. Unlike the adult enzyme, fetal ALA synthetase is not inhibited by the end-product, heme. However, it is more effectively inhibited by high cation concentrations and heavy metals. Other studies are in progress to provide the biochemical and pharmacological basis for predicting and comparing animal and human responses to exposure to porphyrogenic environmental agents.

Doctor Crapo and coworkers have been studying the relationship between the level of superoxide dismutase (SOD) present in lung tissue and the ability of animals to survive 100% oxygen or lethal doses of other pulmonary oxidants. They are attempting to determine the developmental characteristics of the SOD enzyme and to relate these factors to the changing ability of a developing animal to tolerate inhaled oxidants. Rats exposed to nonlethal concentrations of oxygen (85%) for seven days developed "tolerance" to lethal concentrations of oxygen. This ability to tolerate 100% oxygen correlated well with an increased activity of pulmonary SOD. Guinea pigs, hamsters, and mice were also studied but failed to demonstrate either increased activity of pulmonary SOD or acquire tolerance to oxygen. SOD is being characterized and antibody titrations are being used to determine pulmonary levels and different forms of SOD. A large number of environmental toxicants including ozone, NO_2, and paraquat may produce part of their toxic effects by way of the superoxide free radical. Current studies with SOD and oxygen could have broad applications with regard to this type of chemical.

Doctors Weissberg and Crapo have initiated studies of the effects of toxic concentrations of oxygen upon red cell 2,3-diphosphoglycerate metabolism in rats. 2,3-DPG is known to decrease the affinity of hemoglobin for oxygen by selectively binding to deoxyhemoglobin. Elevations in red cell 2,3-DPG with resultant enhanced availability of oxygen to tissues have been reported in instances of lowered arterial oxygen tension. This study attempts to understand the pathophysiologic mechanisms involved in oxygen toxicity.

Doctor Voytek, Doctor Moustafa, and coworkers have initiated studies to establish direct evidence for DNA repair mechanisms in primary mouse oocytes and zygotes to assess the effects on the repair process of physical and chemical agents known to be cytotoxic to mammalian cells. Doctor Voytek and Doctor Clive, of the Environmental Mutagenesis Branch, are also investigating whether thymidine kinase activity in various thymidine kinase mutant cells is caused by mutations in control or structural genes. If structural changes are evident, studies of the physical and kinetic parameters of the enzymes will be carried out.

Mammalian Tissue Culture. Doctor Spalding has made significant progress in his attempts to develop a viable culture system of mammalian gonads for the purpose of investigating the mechanisms by which chemical agents affect oogenesis and spermatogenesis. Doctor McLachlan works closely with Doctor Spalding on this project. Oocyte maturation in ovary explants taken from embryos at 16 and 17 days of gestation was followed for 12-14 days in culture. Oocyte maturation in vitro closely paralleled maturation in vivo up to 25 days post-conception. Twelve-day fetal ovaries were grown in organ culture in order to observe oogenesis in vitro. Growth and maturation of the 12-day explants

paralleled growth in vivo for 6-7 days in culture. Doctor Spalding is also interested in and is studying the application of tissue culture techniques in an attempt to develop more rapid test systems to predict chemical toxicity in man. Techniques of mammalian tissue culture are also being used to study mechanisms of chemical toxicity.

Heavy Metal Toxicology. Doctor Fowler is attempting to define the early ultrastructural effects of heavy metals and other environmental toxicants on mammalian and marine invertebrate tissues. These subcellular morphologic observations are correlated with concentrations and chemical alterations in blood and viscera. His studies attempt to define the ability of electron microscope techniques to predict early signs of chemical toxicity. Arsenic, cadmium, methyl mercury, inorganic mercury, and TCDD are being studied. X-Ray microanalysis techniques are being used which allow localization of heavy metals within cells. Comparative studies of marine organisms allow an estimation of toxicity in the normal setting with natural conditions, a comparison of effects with mammalian species, and a better understanding of the localization and accumulation of these elements by lower animal species.

Membrane ATPase. Doctors Wilson and Sharp have continued to develop techniques to purify beef brain ($Na^+ + K^+$) ATPase and to study the selectivity among phospholipids for stabilizing membranal ($Na^+ + K^+$) ATPases. A more recent project attempts to determine whether and to what extent plasma membranal properties of fetal brain resemble those of the adult. Other studies will determine to what extent alterations in plasma membranal structure and function may correlate with fetal toxicity and/or teratogenic responsiveness. Conditions which would accentuate teratogenic responsiveness to pantothenic acid and/or essential fatty acid deficiencies will be tested. Teratogenic responsiveness to a dietary deficiency and to substitution of vitamin analogs in the diet will be compared. Pregnant rats on a pantothenic acid deficient diet produced fetuses without gross morphologic abnormalities. In contrast, rats on a diet deficient in pantothenic acid and essential fatty acids produced fetuses with a significant number of gross abnormalities and a high incidence of fetal resorptions.

Marsupial Program. Doctor Jurgelsky has continued his studies to evaluate the marsupial as a model for carcinogenesis. The autopsy phase of this project has been completed. Ethyl nitrosourea, a known potent carcinogen, was administered orally as a single dose (100 mg/kg) to opossums at 10 different stages of development. Eye tumors and several other unusual tumors have been found in ENU-treated animals which might be of interest to the National Cancer Institute.

Summary. The Environmental Toxicology Branch will make a greater effort in the coming year to coordinate the efforts of all Branch scientists according to "strategic plans" which are being developed currently. Broad basic and applied questions will be defined as Branch goals. Research efforts will be directed to accomplishing the defined steps of a flexible plan to reach these goals most efficiently and effectively. Major areas being organized are (1) detection of the teratogenic potential of environmental chemicals, (2) use of in vitro techniques in toxicology, (3) in vitro gonadogenesis and fertilization, (4) reproductive effects and toxic mechanisms of gestational and postnatal chemical exposure, and (5) development of more effective biochemical and morphologic

test procedures for chronic chemical toxicity. It is important to define our Branch goals somewhat more narrowly than in the past to be most effective with the limited personnel and fiscal resources currently available.

Serial No.: NIEHS-ET-007
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis"

PREVIOUS SERIAL NUMBER: NIEHS-PP-032

PRINCIPAL INVESTIGATORS: I. P. Lee, Ph.D.

OTHER INVESTIGATORS: R.-S. Shen, Ph.D., K. Okumura, Ph.D., S.-W. Kwan, Ph.D., R. L. Dixon, Ph.D.

COOPERATING UNITS: Hazleton Laboratories, Inc.

MAN YEARS

Total:	3.3
Professional:	2.5
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate unscheduled DNA synthesis in mouse spermatogenic cells as a possible new predictive test for screening environmental mutagens using in vitro and in vivo techniques; (2) to study synthesis and structure of messenger RNA in rat spermatogenic cells; (3) to define enzyme development during spermatogenesis and attempt to develop useful "marker enzymes" to indicate normal cellular differentiation and toxic effects using velocity sedimentation cell separation and serial mating; and (4) to investigate the nature of the blood-testis barrier and determine the role of this permeability barrier in the penetration of various environmental chemicals to testicular fluid.

METHODS EMPLOYED: In vitro DNA repair was studied by incubating isolated seminiferous tubules with varying concentrations of mutagens. Tritiated thymidine was added subsequently and incubation continued. Spermatogenic cells were separated by velocity sedimentation cell separation and the incorporation of thymidine into various cell types was quantitated.

Mice were injected with varying doses of mutagens to determine DNA repair in vivo. Seminiferous tubules were isolated and cells separated. The amount of thymidine incorporated by specific spermatogenic cell types was quantitated.

Dominant lethality studies using mice were conducted using the following mutagens: MMS, IPMS, TEM, Cytoxan, Busulfan, and Mitomycin-C.

DNA repair enzymes: DNA polymerase enzymes were determined according to the modified method of Wicha and Stockdale (BBRC 48: 1079, 1972).

Preparation of polysomal RNA from rat spermatogenic cells: Spermatogenic cell cytoplasm was isolated by treating isolated minced seminiferous tubules with a non-ionic detergent, triton X-100, to remove nuclei. mRNA was labeled with tritiated adenosine and total polysomal RNA extracted by the usual phenol method. For the isolation of mRNA, the differential phenol extraction procedure was followed by binding to Nitrocellulose Membrane filters (Brawerman et. al., Biochem. 11: 637, 1972). Sucrose density gradient analysis showed a typical size distribution profile of polysomes with little contamination of small messenger ribonucleoprotein particles.

Determination of spermatogenic cell enzymes: Semininiferous tubules were isolated from postnatal mice at varying ages after birth and homogenized. Enzyme activities of hyaluronidase (H), carbonic anhydrase (CA), sorbitol dehydrogenase (SDH), glyceraldehyde-3-phosphate dehydrogenase (GSPH), α-glycerophosphate dehydrogenase (GPDH), glucose-6-phosphate dehydrogenase (G6PDK), lactic dehydrogenase (LDH), lactic dehydrogenase X (LDH-X), isocitrate dehydrogenase (ICDH), and malic dehydrogenase (MDH) were assayed according to the usual methods.

Preparation of rat testis for blood-testis barrier studies: Efferent ducts of rat testes were ligated 18 hours before cannulating the rete testis using microcapillary tubes. Rete-testis fluid was collected and various compounds administered to study their pharmacokinetic parameters. Constant infusion of H_2O, inulin, galactose, urea, sulfonamides, and barbiturates were used to establish blood levels and to characterize the permeability of blood-testis barrier in terms of molecular size, pk_a, and lipid solubility of the test chemicals.

MAJOR FINDINGS:

(1) DNA Repair in Spermatogenic Cells:

a. Methylmethane Sulfonate (MMS). Both in vitro and in vivo exposure of spermatogenic cells to varying doses of MMS followed by velocity sedimentation cell separation demonstrated that radioactivity (thymidine) was associated with spermatogenic cells sedimenting at 15.1~17.1 and 5.4 mm · H_R^{-1}, respectively. These acid insoluble radioactive DNA peaks represent premeiotic and spermatogonial cells. DNA repair activity was optimal at 16 hours after the administration of MMS at a dose of 16.25 mg/kg. However, at 32.5 and 65 mg/kg doses of MMS, there was no detectible DNA repair up to 48 hours after treatment. Therefore, DNA repair activities appear to be biphosic and dose dependent.

b. Isopropylmethane Sulfonate (IMPS). DNA repair activity was optimal at 37.5 mg/kg body weight and at 16 hours after a single treatment of this compound. At higher doses, 75 and 150 mg respectively, no repair activities were observed.

c. Dominant Lethal Studies. Studies with MMS, IPMS, Busulfan, TEM, Cytoxan and Mitomycin-C demonstrated that the major spermatogenic cell types affected by these agents were as follows: MMS (spermatogonia and spermatids), IPMS (spermatogonia and early premeiotic cells), Busulfan (spermatozoa), Cytoxan (spermatozoa and spermatids), TEM (spermatozoa and spermatids), and Mitomycin-C (premeiotic cells). Extent of dominant lethalities was a dose dependent phenomena.

d. Effects of Cadmium on DNA Polymerase Activities. Effects of cadmium on testicular DNA polymerase activity were determined after a single treatment. Mice received 0.5, 1.0, 2.0 and 4.0 mg/kg of cadmium as a function of time. The maximum inhibition of DNA polymerase was 5 days after treatment with 0.5 mg/kg (84% of control); 11 days after treatment with 1 mg/kg (71% of control); 20 days after treatment with 2 mg/kg (62% of control), and 20 days after treatment of 4 mg/kg (51% of control), respectively. Pretreatment with zinc 19 hours prior to cadmium treatment did not alleviate the effects of cadmium on testicular DNA polymerase.

(2) Messenger RNA Synthesis in Rat Spermatogenic Cells. Testes from 9-22 day-old rats were found to actively synthetize both rRNA and mRNA. Like most mammalian mRNA, that from the early spermatogenic cells has heterogeneous sedimentation characteristics and was shown to contain a poly (A) sequence. Preliminary experiments suggest that this poly (A) segment is present in almost all mRNA molecules.

(3) Spermatogenic Cell Enzymes. Determinations of various enzymes were carried out with postnatal mice as a function of age. Enzyme activities of SDH, LDK, LKH-X, Hyaluronidase (H), and α-GPDH were increased at least 5 to 10 fold in their respective enzyme activities about 30 days after birth. When mice were treated i.p. with 400 mg/kg of procarbazine, the disappearance of major spermatogenic cell types correlated well with the diminution of LDH, LDH-X, SDH, α-GPDH, and Hyaluronidase. Similarly, lowered G-6-P dehydrogenase activity correlates well with a decreased number of spermatogonia and spermatocytes.

(4) Blood-testis Barrier. The permeability of the blood-testis barrier has been studied, and transfer rate constants were derived from Davson's equation. The results showed that the correlation coefficient between partition coefficient and transfer rate constants of compounds was 0.95 and very significant ($p<0.01$). The most important factor for permeability of compounds across blood-testis barrier was lipid solubility. This barrier was found to be similar to blood-brain barrier. Transport of cadmium ion appeared to be an active process. Zinc pretreatment inhibited the uptake of cadmium ion into rete-testis fluid.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies of toxic effects of environmental agents on reproduction in the past centered largely on teratogenicity and dominant lethal studies. Few investigations have been concerned with the effects of chemicals on fertility and their possible mechanisms of toxic action. Understanding of DNA repairability in spermatogenic cells will facilitate the evaluation of dominant lethality based on more scientific rational. Studies of spermatogenic enzyme profiles associated with

spermatogenic cell differentiation and aging will not only help to explain the mechanism of toxic effects, but will also be useful as "marker enzymes" in predicting the type of spermatogenic cells that are damaged by toxic agents.

Studies of the blood-testis barrier are important with respect to understanding how different chemicals can get across and get into spermatogenic cells.

PROPOSED COURSE: Studies will be continued to determine extent of DNA repair in spermatogonial cells and to relate this phenomena to dominant lethal effects. Further studies regarding toxic effects of environmental agents on DNA repair enzymes will be conducted. Other experiments are underway to determine the size of the poly (A) sequence and to further characterize the mRNA in terms of kinetic parameters.

Future studies of the blood-testis barrier will involve perfusion techniques. Attempts will be made to define the interrelationship of these basic mechanisms and prediction of the toxic effects of environmental chemicals.

PUBLICATIONS

Lee, I. P. and Dixon, R. L.: Effects of cadmium on spermatogenesis studied by velocity sedimentation cell separation and serial mating. J. Pharmacol. Exp. Ther. 187: 641-652, 1973.

Shen, R. S. and Lee, I. P.: Procarbazine-induced testicular enzyme changes. The Pharmacologist 15: 163, 1973.

Dixon, R. L., Okumura, K., and Lee, I. P.: Studies of the blood-testis barrier with regard to the toxicity of environmental agents. The Pharmacologist. 15: 163, 1973.

Serial No.: NIEHS-ET-002
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effect of Environmental Chemicals on Oogenesis and Ovarian Function in Mammals"

PREVIOUS SERIAL NUMBER: NIEHS-PP-033

PRINCIPAL INVESTIGATORS: J. A. McLachlan, Ph.D.

OTHER INVESTIGATORS: R. L. Dixon, Ph.D., J. W. Spalding, Ph.D., and H. C. Shah, Ph.D.

COOPERATING UNIT: None

MAN YEARS

Total:	2.8
Professional:	2.0
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: (1) To evaluate the effects of prenatal exposure to environmental chemicals on the subsequent reproductive capacity of the offspring, (2) to investigate the mechanisms involved in the production of subfertility in females as a result of their *in utero* exposure to foreign chemicals, (3) to assess the transplacental carcinogenic potential of these compounds, and (4) to develop an embryonic mouse organ culture system for the study of oogenesis *in vitro*.

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the functional status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, quantity and morphology of ova obtained from the female reproductive tract, and histopathologic evaluations of the ovary including quantitative determinations of the various follicular classes. Routine biochemical, physiologic, and morphologic procedures were used which include polyacrylamide gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, and microdissection. Standard organ culture techniques were employed to maintain explants of fetal mouse ovaries.

MAJOR FINDINGS:

Effect of prenatal exposure to DES on the reproductive capacity of the offspring: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock food additive and gynecologic medication. Experiments in our laboratory suggest that prenatal exposure to DES adversely affects the reproductive capacity of the female offspring. Timed pregnant mice were given DES during the period of ovarian development (days 10 through 16 of gestation) in doses ranging from 0.01 to 100 µg/kg. When the female offspring of these mice reached sexual maturity, they exhibited varying degrees of subfertility ranging from a 40% reduction in total reproductive capacity (0.01 µg/kg dose) to sterility (100 µg/kg dose).

No differences were observed between control or DES-exposed offspring (at any dose level) with regard to estrous cycles or the distribution of follicular types in their ovaries. However, following induction of superovulation, there was an 80% reduction in the number of ova recovered from the oviducts of female mice exposed prenatally to the high dose of DES.

Reciprocal transplantation studies in which fertilized ova from DES-treated females were transferred to the uterus of control females and the corresponding control conceptuses were transplanted into the uterus of DES-treated offspring were performed to evaluate the relative contributions of the oocyte and uterus to the observed reproductive failure.

In vitro Oogenesis: Experiments were recently begun to isolate, explant, and culture in chemically-defined media the ovarian primordia of fetal mice. Explants of 12 day (premeiotic oocyte stage) and 16 day (postmeiotic oocyte stage) fetal ovaries were maintained in organ culture for one and two weeks, respectively. Observed morphological changes during these limited culture times correlated well with those seen in vivo during the corresponding periods. The further improvement of the mouse embryonic gonad organ culture system will provide information on basic processes of oogenesis or folliculogenesis and, in addition, may provide a screening technique for the ovotoxic potential of environmental compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although many compounds are continuously introduced into our environment, few of them have been examined for the potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such in utero drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic damage to the developing oocyte, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PROPOSED COURSE: The studies dealing with the total reproductive capacity of mice exposed to diethylstilbestrol in utero will be continued with emphasis placed on determining the basic mechanisms underlying the observation. Experiments to determine sensitive periods and structure-activity relationships

involved in this effect will be undertaken. Moreover, studies of steroid and/or gonadotropin receptors in the prenatally DES-exposed females will be attempted. The mouse embryonic gonad organ culture system will be refined by initally studying follicle kinetics and oocyte DNA synthesis *in vitro*.

PUBLICATION

McLachlan, J. A. and Dixon, R. L.: Effect of gestational exposure to DDT or diethylstilbestrol on the reproductive capacity of offspring. *The Pharmacologist*. 15: 199, 1973.

Serial No.: NIEHS-ET-003
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effect of Environmental Components on Mammalian Ontogenesis After Parental Exposure"

PREVIOUS SERIAL NUMBER: NIEHS-PP-044

PRINCIPAL INVESTIGATORS: R. E. Staples, Ph.D., F. D. Andrew, Ph.D., S. N. Baksi, Ph.D., C. A. Kimmel, Ph.D., M. H. Lu, Ph.D., R. R. Maurer, Ph.D., and L. A. Moustafa, Ph.D.

OTHER INVESTIGATORS: G. F. Dean, A.A.S., E. H. Floyd, R.T., R. G. Kellam, B.S., S. B. McCaffity, J. P. Putnam, B.A., F. L. Schwartz, M.S., C. D. Sloan, M.S., and P. E. Watkins, M.T. (ASCP)

COOPERATING UNITS: Research Triangle Institute (Procurement Contract: NIH-73-C-586). Environmental Biophysics Branch (Noise). Pharmacology Branch (Adhesives).

MAN YEARS

Total:	6.2
Professional:	3.6
Other:	2.6

PROJECT DESCRIPTION

OBJECTIVE: To determine the developmental toxicity and teratogenic potential of single or combined environmental components in mammals by application of the agents (a) to the dam and (b) by local application to the developing conceptus *in vivo*.

METHODS EMPLOYED: Test agents were given to pregnant females of at least two species, e.g., mouse and rabbit by two or more routes, e.g., per os, subcutaneous, intrauterine, for various periods of ontogenesis. Doses were selected on the basis of preliminary study. Those which produce a given pharmacological response (maximum tolerated dose, comparable blood levels, 50% fetal mortality, etc.) were selected. Stresses or disease states that may be elicited before mating were also studied for teratogenic potential either alone or in combination with other environmental components. An attempt was made to compare the sensitivity of various approaches. Qualitative or quantitative differences or similarities in teratogenic response were noted. Resulting offspring were examined for alterations in the incidence of external, visceral, and skeletal changes shortly before term. When indicated, both males and females can be

exposed to the test agent before breeding and the offspring may be studied at other stages of gestation for morphologic changes. Postnatal studies determine differences from controls with respect to survival, growth rate, behavioral changes, and functional capability. After exposure of males and females to test agents, various reproductive parameters were examined for impaired function when indicated by the results of preliminary studies.

MAJOR FINDINGS: Provera (medroxy progesterone acetate), which has been categorized as a possible teratogen in humans, was evaluated as a teratogen in rats, mice, and rabbits. Embryotoxicity was not observed in mice and rats even at the highest test dose (300 mg/kg) but stunting, cleft palates, and some other malformations were seen among the offspring of rabbits given 3 and 10 mg/kg/day for 3 days during organogenesis. Palate malformations are very rarely observed in control rabbits.

Female rats were thyroparathyroidectomized at 24, 45, or 75 days of age and were subsequently bred at about 90 days of age. About one-half of the young of each litter died before term. However, there was no evidence of a teratogenic response among the survivors after evaluation of the offspring for external, visceral, and skeletal alterations. Some rats thyroidectomized at 75 days of age and similarly bred at about 90 days of age were treated with dichlorvos (25 mg/kg) during organogenesis. Dichlorvos was not demonstrated to be teratogenic in fetuses from euthyroid or thyroidectomized female rats and no potentiation of toxicity was evident in thyroidectomized animals.

Thyroidectomy was performed on the offspring of rats between 4 and 21 days postpartum; a critical period for brain development. It is now possible to maintain a large proportion of these young beyond the nursing period, thereby providing a model for studying subsequent development and the reproductive potential of individuals thyroidectomized at an immature state of development.

To determine the teratogenic effects of noise, mice (C.F.S.) were housed in a sound-proof chamber throughout gestation. Reduction of noise to the level generated by the animals themselves, about 40dBA during daylight and 55dBA during darkness, did not significantly alter the incidence of malformations. Hence, the noise level in mouse quarters *per se* is not likely inducing malformations in untreated mice.

Subcutaneous administration of captan to mice at dosages up to 100 mg/kg body weight from Days 6 through 15 of gestation did not adversely affect fetal survival or development. Intrauterine administration of captan at 300 µg/uterine horn on Day 8 also was not embryotoxic; however, similar administration on Day 12 resulted in significant fetal lethality, but teratology was not produced.

Tenuazonic acid, a mycotoxin extracted from rice infected with Alternaria, was administered to mice orally for various intervals between Days 8 and 15 of gestation. A teratogenic response was obtained after single administration (66 to 200 mg/kg) on Days 8 or 10, but not on Day 12 of gestation. Terata were not evident upon injection of the crude extract at doses as high as 2000 mg/kg on Day 10 of gestation.

A spray adhesive was administered to rats, mice, and hamsters on Day 8, 10, or 12 of gestation by inhalation. Neither embryotoxicity nor teratology resulted even at doses which significantly reduced maternal weight gain for two days after treatment in both rats and mice.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Through the efforts described, the teratogenic potential of environmental components are evaluated. The teratogenic response of various species to some of the agents being tested *in vivo* is compared to the response after exposure of preimplantation embryos during culture. Our hope is that a means of testing can be derived that will improve extrapolation of experimental results to man by circumventing variable maternal pharmacokinetic actions which affect the exposure of the embryo to the test agent.

Modern man is exposed continuously to a multitude of physical stresses and chemical insults, yet most agents tested for teratogenic potential are still being studied as single entities even though synergistic effects in animals have been documented. One of the goals of this study is to determine the likelihood of synergism of teratogenic potential with combinations of environmental components to which the housewife might reasonably be exposed. Environmental components to be tested are first studied individually and then, depending upon the information gained, combinations of components will be tested.

A second goal of this study is to determine possible merits of local application of test agents. Perhaps variability of response to teratogens can be reduced among species if maternal pharmacokinetics are circumvented in this manner. Teratogenic agents might also be identified after local application of only a small fraction of the dose currently required by *in vivo* tests.

The type of question we seek to answer is that typified by the situation with Provera. Is Provera likely to produce malformations in man? Would all three species tested give positive results if the Provera were injected into the uterus? Would a hypothyroid woman be at increased risk?

PROPOSED COURSE: (1) To test selected agents by local application to enable route comparision, e.g., Provera, Captan, Dichlorvos, DMSO, DMS. (2) To test congeners of Provera in the rabbit in an attempt to identify a structure activity-relationship; to extend exposure in rats and mice to Provera for nine days rather than the three days tested to date. (3) To test the teratogenic potential of increased noise levels. (4) To determine whether the young of thyroidectomized animals are more susceptible to Provera than are the young of euthyroid female.

Serial No.: NIEHS-ET-004
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Survival and Development of Mammalian Gametes and Embryos After In Vitro Exposure to Selected Chemicals and Conditions"

PREVIOUS SERIAL NUMBER: NIEHS-PP-041

PRINCIPAL INVESTIGATORS: R. E. Staples, Ph.D., R. R. Maurer, Ph.D., and L. A. Moustafa, Ph.D.

OTHER INVESTIGATORS: E. L. Floyd, R.T., R. G. Kellam, B.S., F. L. Schwartz, M.S., and W. Willis, M.S.

COOPERATING UNITS: Research Triangle Institute (Procurement Contract: NIH-73-C-586) and Environmental Biophysics Branch (Microwaves).

MAN YEARS

Total:	2.8
Professional:	1.1
Other:	1.7

PROJECT DESCRIPTION

OBJECTIVE: To determine the effects of various chemical and physical entities, acting alone and in combination, on subsequent development of mammalian gametes and embryos after exposure in vitro.

METHODS EMPLOYED: Gametes and embryos were recovered from donor animals and cultured in vitro for varying periods. Chemicals or conditions being tested were generally applied during the culture period. On occasion the donor may have been exposed and the treated gametes used for fertilization in vivo or for undergoing in vivo fertilization followed by additional culturing. Some preimplantation embryos were transferred to recipient females while others were examined for morphologic or chemical changes. Transferred embryos were usually examined near term for external, visceral, and skeletal malformations, but when indicated, they had been studied at earlier stages of gestation or even following birth.

MAJOR FINDINGS: Dimethyl sulfoxide (DMSO) and its metabolite, dimethyl sulfone, were added to cultures of mouse blastocysts for six hours, at maximum concentrations compatable with survival, i.e., 8.5% and 5.0% final concentration, respectively. Surviving blastocysts were transferred to recipient females. A teratogenic response was not obtained after exposure to either agent when the fetuses were evaluated near term. Previous studies have demonstrated DMSO to

be teratogenic in the mouse after application by the intraperitoneal route, if not also by the subcutaneous and oral routes. Teratogenicity has not yet been demonstrated after administration of DMSO by the intrauterine route.

Effects of microwave radiation on developing mouse embryos was studied in the absence of a simultaneous increase in heat. Partitioning of the possible effects of heat from those of the microwave radiation per se was achieved by using a power level that would maintain an in vitro culture system at 37.5°C. After continuous exposure for 22 hours at a power level of 100 mW/cm^2 at 2450 MHz, the embryos were transferred to recipient females. A teratogenic response was not detected when the surviving fetuses were examined for malformations near term.

Tenuazonic acid, a mycotoxin of Alternaria, was added to in vitro cultures of mouse morula as the sodium salt at a concentration of 20 µg/ml. This concentration was the maximum that could be added without inhibiting cleavage. After exposure for 2, 24, or 48 hours, the embryos were transferred to recipient female mice. A teratogenic response was not detected among the fetuses that survived to time of sacrifice day 18 of gestation. Administration of the tenuazonic salt to pregnant mice by the oral route between Days 8 and 10 of gestation was teratogenic.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Techniques permitting development of preimplantation embryos in vitro with retention of viability to term after transfer to the uteri of recipient females makes possible direct exposure of mammalian embryos to test agents, thereby permitting the investigator to distinguish between direct and indirect effects of environmental insults on embryo development. Information will also be gained that will increase our understanding of embryonic requirements for development. Results of exposure by this route are compared to those obtained by administration to the dam and to the uterus with regard to detecting differences in response.

PROPOSED COURSE: To study effects of captan, dimethyl sulfide, sulfur dioxide, and selected heavy metals on subsequent development through use of mouse, rat, and rabbit embryos.

PUBLICATIONS

Elliot, D. S., Maurer, R. R., and Staples, R. E.: Development of mammalian embryos in vitro with increased atmospheric pressure. Biol. Reprod. In Press.

Serial No.: NIEHS-ET-005
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Interaction of Complexing Agents with Heavy Metals and Effects on Teratogenicity"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: C. A. Kimmel, Ph.D.

OTHER INVESTIGATORS: C. D. Sloan, M.S. and G. F. Dean, A.A.S.

COOPERATING UNITS: None

MAN YEARS

Total:	1.9
Professional:	0.7
Other:	1.2

PROJECT DESCRIPTION

OBJECTIVE: To investigate metal chelation as a mechanism of teratogenic action by using various known chelators and studying their interactions with essential and toxic heavy metals.

METHODS EMPLOYED: All animals were maintained during pregnancy on a purified diet of known composition to eliminate variability of heavy metal contamination in commercial chow. The interaction of EDTA or other known chelators such as salicylates with zinc and other essential metals was studied using oral or subcutaneous administration of chelators in an attempt to alleviate metal toxicity during pregnancy. Offspring of animals in these studies were examined for teratogenic effects just before term on a contract with Research Triangle Institute.

MAJOR FINDINGS: Oral intubation or subcutaneous administration to rats of large doses of EDTA for up to 6 days of gestation produced few malformations in contrast with the work of Swenerton and Hurley who administered lethal levels of EDTA in the diet. EDTA has low teratogenic potential. Cadmium or lead, even after administration of high doses for five days, by oral intubation or subcutaneous administration failed to produce significant terata. In those animals in which cadmium had a teratogenic effect, there appeared to be a correlation with decreased food consumption during and following treatment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many chelating agents are found in the environment (i.e., as natural products, food preservatives, and pollutants) and may have significant effects in areas where essential metal or protein deficiencies occur. The interaction of such chelators with essential metals could produce a temporary metal deficiency and result in inhibition of enzymes necessary for critical stages of embryonic development. The use of chelators to alleviate heavy metal toxicity during pregnancy will become more important as heavy metal contamination increases in our environment. These studies may provide useful information concerning possible risks associated with chelation therapy.

PROPOSED COURSE: We will continue to look for teratogenic effects with EDTA, cadmium, or lead using long-term exposures in the diet or water. Absorption, distribution, and excretion of compounds will be determined and their effects on both embryonic and perinatal enzyme systems will be studied for correlation with teratogenic effects.

Serial No.: NIEHS-ET-006
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Low Temperature Storage of Mammalian Embryos"

PREVIOUS SERIAL NUMBER: NIEHS-PT-026 and NIEHS-PP-042

PRINCIPAL INVESTIGATOR: R. R. Maurer, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D., J. K. Haseman, Ph.D., F. L. Schwartz, M.S., H. L. Bank, Ph.D., Department of Anatomy, Duke University, and L. C. Ulberg, Ph.D., Department Animal Science, North Carolina State University

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS

Total:	0.6
Professional:	0.4
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: (1) To establish methodology for the storage of viable mammalian embryos, (2) to assess the effects of freezing and low temperature storage on subsequent *in vitro* and *in vivo* development, (3) to determine if mammalian embryos of various species respond similarly to freezing and low temperature storage, and (4) to provide a bank of stored embryos.

METHODS EMPLOYED: Embryos are recovered by conventional methods from mice on Day 3 of gestation and from rabbits on Day 2 or 3 of gestation. The embryos are washed with and placed in a phosphate buffered saline solution fortified with pyruvate, bovine serum albumin and DMSO. Embryos are frozen slowly (0.5 to 1.5°C/min), and the temperature gradually reduced to -100°C before the embryos are placed in liquid nitrogen (-196°C). After various periods of storage, the embryos are thawed slowly (4 to 50°C/min), washed and placed in culture for 48 hours. After culturing, the resulting mouse blastocysts are transferred to the uterus of Day 3 recipient mice. Some of the recipients are allowed to parturate. The fetuses from the remaining recipients are removed by Cesarean section one or two days before normal delivery and examined for teratological changes. Rabbit morula are transferred immediately after thawing to Day 3 recipients for subsequent evaluation of *in vivo* development.

MAJOR FINDINGS: Mice: eight-celled embryos stored for 1 or 7 days at -196°C did not differ with respect to *in vitro* development, however freezing did reduce the number of embryos which developed a blastocoele compared to culture controls. *In vivo* embryo survival was reduced by culturing and further reduced by freezing. The reduction resulted from a slight decrease in the pregnancy rate for females receiving frozen embryos. No increase in teratogenic alterations was noted in any of the groups.

Freezing embryos had virtually no effect on weights from birth to 65 days of age. Other body measurements were similar to control groups. Reproductive capability of mice frozen as embryos was similar to control groups and postnatal development of the f_1 progeny was similar to respective control progeny.

Storage temperature was found to be much more important than storage duration. Embryos should be stored at -196°C or lower. Viable young have been obtained after being stored as 8-celled embryos at -196°C for 1 year.

Rabbit: Eight-celled and morula stage rabbit embryos have also been frozen and thawed successfully as judged by subsequent *in vitro* and *in vivo* development. Rabbit fetuses which developed from frozen embryos had no observable adverse effects from freezing. Freezing parameters used to successfully freeze embryos differed for species as well as for the different stages of embryonic development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Human spermatozoa are frozen and banked for routine clinical inseminations as well as for vasectomized males who may at a later time desire children. The eventual storage of human embryos must also be considered. The main cryoprotective agent is DMSO, which is known to be teratogenic at high levels. Therefore, the potential hazard should be assessed through use of animal models.

The freezing and storage of embryos can aid experimentation by providing a readily available source of material, preserving mutant strains of species, and providing a more precise measurement of genetic drift as well as changes in susceptibility to various environmental insults in mammalian species and strains. Proper preservation of embryos will reduce preparation artifacts, thereby aiding subcellular studies. The general technique being developed can also provide further information regarding the freezing and banking of human tissues and organs.

PROPOSED COURSE: The freezing technique will be utilized in additional studies. Species differences will be further investigated as embryos become available, i.e., swine embryos.

Serial No.: NIEHS-ET-007
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Uterine Luminal Secretions and Embryonic Development"

PREVIOUS SERIAL NUMBER: NIEHS-PP-046

PRINCIPAL INVESTIGATOR: R. R. Maurer, Ph.D.

OTHER INVESTIGATORS: H. M. Beier, Ph.D., Anatomical Institute, University of Kiel, W. Germany, and F. L. Schwartz, M.S.

COOPERATING UNIT: Environmental Biometry Branch

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the biologic significance of uterine luminal secretions present during early pregnancy, and (2) to determine whether quantitative or qualitative changes in luminal secretions occur when animals are placed under stress or subjected to environmental factors.

METHODS EMPLOYED: The effects of uterine luminal proteins on cultured embryos were studied. The effects of these proteins which were isolated from pseudo-pregnant (5-6 days) rabbits were compared to those associated with the proteins which are normally part of the incubation medium. In addition, rabbit uterine luminal fluids were collected on Days 4 to 6 of gestation and the proteins were examined electrophoretically to determine the normal composition and to determine the effect of various environmental factors on these constituents. Radioimmunoassay procedures were used to determine steroid concentrations in the uterine luminal fluids.

MAJOR FINDINGS: Embryonic development requires close synchrony between the developmental stages of the uterus and the embryo. This necessity for synchrony is also required in *in vitro* systems. Eight-celled rabbit embryos cultured for 96 hrs failed to develop whereas morula stage embryos continued to develop when uterine luminal proteins collected on Day 5 of gestation were used as the macromolecular component of the culture medium. Rabbit uterine prealbumin, in contrast to uterine albumin, uteroglobin, and glycoprotein reduced blastocoele development of morula stage embryos. Uterine luminal proteins were found

to bind differing ratios of progesterone to other progestins. Estrogen levels were less than 10 pieograms per milligram protein. Supplementation of the culture medium with levels of progesterone and estrogen encompassing those found in the luminal proteins did not affect blastocoele formation. Progesterone reduced blastocyst expansion. Prostoglandin $F_{2\alpha}$ levels of 10^{-2} to 10^{2}M did not reduce blastocoele formation but did reduce blastocyst expansion.

Quantitative and qualitative differences in luminal proteins were detected with electrophoretic methods among 4 to 6 day pregnant rabbits. Injection of progesterone prior to ovulation elicited a premature uteroglobin appearance in the luminal protein, thereby changing the uterine-embryo synchrony.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elucidation of the biologic significance of uterine secretions would add to our knowledge of the processes necessary for the further development of preimplantation embryos. If environmental components can be shown to alter the composition of uterine secretions, additional experimental efforts might demonstrate such alterations to be at least partially responsible for the high level of embryonic mortality seen in virtually every mammalian species at the time of implantation. These findings might also be of importance in the development of newer methods for population control.

PROPOSED COURSE: Uterine luminal constituents will be tested further to seek a component that will initiate or inhibit blastocoele formation. Uterine luminal proteins obtained from control and stressed females will be examined for qualitative and quantitative differences.

Serial No.: NIEHS-ET-008
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effect of Direct Application of Test Agents to the Mammalian Embryo on Subsequent Viability and Development"

PREVIOUS SERIAL NUMBER: NIEHS-PP-043

PRINCIPAL INVESTIGATOR: L. A. Moustafa, Ph.D.

OTHER INVESTIGATORS: R. E. Staples, Ph.D., and W. D. Willis, B.S.

COOPERATING UNITS: None

MAN YEARS

Total:	0.8
Professional:	0.4
Other:	0.4

PROJECT DESCRIPTION

OBJECTIVE: To investigate the susceptibility of the developing mammalian embryo to test chemicals applied directly.

METHODS EMPLOYED: A micrurgical technique developed by the principal investigator was adapted for direct administration of test agent(s) (e.g., Thalidomide) into mammalian blastocysts. After micromanipulation, blastocysts were prepared for study by histochemical and cytological techniques or were transferred into recipient females. Pregnant females were sacrificed prior to natural term for fetal evaluation. Preimplantation embryos were also exposed to the test agent(s) during culture period prior to transfer into recipient females and/or cytological evaluation. Radiolabeled compounds were used to follow the uptake of tested agents. Results were compared to those obtained after *in utero* (microsurgical) application of the same test agents.

MAJOR FINDINGS: A micrurgy system was established for direct administration of foreign chemicals to mouse and rabbit preimplantation embryos. Embryos were micromanipulated, cultured, and transferred into foster mothers with no apparent adverse effects on development. Accumulated data indicated that the applied thalidomide dose was embryolethal only; lower doses are under investigation to determine whether malformation will result (in rabbit).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The technique permits study of the direct effects of various compounds on the

developing mammalian embryo. It is hoped that interspecies variability in embryonic response will be minimized, thereby increasing the predictability of what might be expected of human embryos upon exposure to a given concentration of the parent test agent(s).

PROPOSED COURSE: Investigations will be continued to determine whether direct application of environmental agents and classic teratogens offers any advantage for problems involving teratology with particular regard to increased sensitivity and reduced variability of response among species.

Serial No.: NIEHS-ET-009
1. Environmental Toxicology Branch
2.
3. Research Traingle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Studies of Homotypic and Heterotypic Interactions in Chimaeric Mammals"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: L. A. Moustafa, Ph.D.

OTHER INVESTIGATOR: W. D. Willis, B.S.

COOPERATING UNITS: None

MAN YEARS

Total:	0.7
Professional:	0.4
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVE: (1) To produce chimaeric mammals (e.g., mouse, rabbit); (2) to analyze and estimate the ratio between transplanted and host cells in chimaeric tissues and organs and their spatial arrangement; and (3) to investigate the effect of inhibition of embryonic cell motility and/or cleavage upon subsequent embryonic development (intact treated embryos and/or the isolated cells transplanted into untreated preimplantation embryos).

METHODS EMPLOYED: Operations were divided into three specific areas: (1) chimaerism was induced by a micrurgical technique (Moustafa, L. A. and Brinster, R. L., J. Exptl. Zool., 181: 181, 1972) in which specific types and numbers of cells were isolated and transplanted into preimplanted mammalian embryos; (2) the complex homotypic and heterotypic (cells of like and unlike developmental history and properties, respectively) interactions in the resultant chimaeric embryos were investigated by observing the cell itself (e.g, pigmentation layer of the retina) and/or the cell product (such as hair pigment or electrophoretic enzyme variants); and (3) determination of the concentration and period of exposure of the selected agent(s) (e.g., Cytochalasin B) to inhibit active embryonic cell locomotion and/or control of cleavage. The compound was added to the culture system or injected micrurgically into the mammalian embryos. In all procedures, micromanipulated embryos were cultured until transferred into suitable recipient females or for later observations in vitro. Recipients were scheduled for caesarian section on the last day of gestation. Fetal viability was recorded, gross congenital malformations noted,

and live fetuses placed with lactating foster mothers which had given birth within the previous 24 hours.

MAJOR FINDINGS: Embryonic single-cells were transplanted into rabbit blastocysts. Phenotypic differences in the eye, skin, and coat color evidenced successful participation of transplanted cells in host organogenesis. An additional measure of successful transplantation was the significantly disproportinate sex ratio favoring the male among the experimental young.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE : The analysis of genetic chimaeras has allowed inferences to be made about several otherwise elusive aspects of development including cell determination, division rates, lineage and deployment and the sizes of pools of precursor cells destined to form particular organs and tissues. Of particular concern is whether a transplanted cell from an embryo with high resistance to an environmental agent would somehow transmit this desideratum to a susceptible recipient embryo; also, whether an auto-immune disease of an embryo can be inhibited by transplanting specific types of cells.

PROPOSED COURSE: After obtaining a suitable number of chimaeric mammals, thorough studies relative to host-transplanted cell interactions and animal phenotype will be conducted. In addition, we will investigate the possibility of producing an interspecific chimaera, where antigenic differences between all cells of the two populations may be detected during all developmental stages. These chimaeras may delineate environmental-genetical interactions affecting the teratogenic expression of an agent found to be embryopathic to a specific strain.

Serial No.: NIEHS-ET-010
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Characterization of Hepatic and Extrahepatic UDP-Glucuronyltransferase and N-Acetyltransferase: Perinatal Development, Modifications of Enzyme Activity, and Enzyme Purification"

PREVIOUS SERIAL NUMBER: NIEHS-PT-036, 037, 038, 039

PRINCIPAL INVESTIGATORS: G. W. Lucier, Ph.D. and B. R. Sonawane, Ph.D.

OTHER INVESTIGATORS: O. S. McDaniel, P. A. Singletary, G. E. R. Hook, Ph.D., J. R. Bend, Ph.D., B. A. Fowler, Ph.D., and J. A. Moore, D.V.M.

COOPERATING UNITS: None

MAN YEARS

Total:	3.5
Professional:	2.7
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: (1) Compare activities of UDP-glucuronyltransferase and N-acetyltransferase in liver, kidney, lung, small intestine, placenta, testes, and brain; (2) determine enzyme specificity and possible enzyme multiplicity, for example, whether there are different UDP glucuronyltransferases responsible for the glucuronidation of steroids and of exogenous chemicals; (3) study the balance of deglucuronidation vs glucuronidation reactions in different tissues and at different ages; (4) establish perinatal development patterns of conjugative enzymes in different tissues so that information can be obtained concerning the relative importance of conjugative enzymes in detoxication-regulation pathways in different tissues at different stages of development; (5) purify glucuronyltransferases and N-acetyltransferases so that mechanisms responsible for developmental fluctuations in enzyme activity can be elucidated; (6) discover the biochemical indicators that are associated with the development of enzyme activity; (7) characterize the mechanisms responsible for alterations in activity of conjugative enzymes following exposure to environmental agents such as the chlorinated dibenzodioxins and dibenzofurans.

METHODS EMPLOYED: Microsomes were prepared using standard procedures. Rapid radiometric techniques were developed in this laboratory, to measure glucuronyltransferase and N-acetyltransferase in vitro, based on the partition of

non-polar compounds into non-aqueous scintillation fluid.

Substrates used for glucuronyltransferase assays were 1-naphthol, p-nitrophenol, testosterone, estrone, estriol, and estradiol. Substrates used for N-acetyltransferase were p-aminobenzoic acid, 4-aminobiphenyl, 2-aminofluorine, p-aminosalicyclic acid, Isoniazaid, and Sulfmethazine. Validities of colorimetric methods for assaying enzyme activity were determined by using radiolabeled substrates and resolving product from substrate on DEAE-cellulose columns and thin-layer plates.

In the developmental studies, at least 15 different ages were used so that sudden fluctuations in enzyme activity could be detected and placental activities measured at many gestational periods. Tissues studied were liver, lung, kidney, small intestine, and placenta and species used were the rat, rabbit, and guinea pig.

Purification of N-acetyltransferase, glucuronyltransferase, and β-glucuronidase were accomplished using a variety of procedures; salt fractionation, pH fractionation, density gradients, ion-exchange chromatography, gel filtration, electrophoresis, and iso-electric focusing.

Environmental chemicals (chlorinated dibenzo-p-dioxins and heavy metals) were administered to the mother prior to mating, or as single or multiple doses during different gestational periods representing varying stages of organogenesis. Enzyme activities of maternal, fetal, and neonatal tissues were measured at different time periods during gestation and after birth. Fetal pharmacokinetic studies of the administered chemicals (radiolabeled) were done in conjunction with the enzyme studies.

MAJOR FINDINGS:

Perinatal Development. Perinatal development of P-nitrophenol (PNP) glucuronyltransferase, testosterone glucuronyltransferase, and β-glucuronidase were studied in guinea pig and rat microsomes from liver, lung, kidney, small intestine, and placenta. Glucuronyltransferases are solely microsomal whereas β-glucuronidase is located in the lysosomes as well as the microsomes. Enzyme measurements were conducted under optimum conditions for pH, triton X-100 concentration, and Mg^{++} concentration. Guinea pig PNP glucuronyltransferase was first detected at day 45 and steadily increased till birth (day 70) when enzyme activity markedly increased. Rat and guinea pig liver PNP glucuronyltransferase activity, for the first week after was twice that of adults. Liver testosterone glucuronyltransferase activity in rats and guinea pigs did not appear until just prior to birth and there was no postnatal peak in enzyme activity as observed for PNP glucuronyltransferase. These data, along with competitive inhibition data and in vivo effects of chlorinated dibenzodioxins on enzyme activity, strongly suggest that there are at least two microsomal UDP glucuronyltransferases. Guinea pig kidney, intestine, and lung PNP glucuronyltransferases appeared near birth and there was no postnatal peak in enzyme activity similar to that observed for liver enzyme. Enzyme activities reached adult levels two weeks after birth and there was no detectable microsomal testosterone glucuronyltransferase in kidney, small intestine, and lung.

Guinea pig fetal β-glucuronidase exceeded adult enzyme levels in the small intestine but adult activity was higher than fetal activity in the lung. Fetal small intestine β-glucuronidase had the highest deglucuronidation activity of any tissue tested. Liver and kidney β-glucuronidase activities were approximately the same in the respective guinea pig fetal and adult tissues. Deglucuronidation (β-glucuronidase) predominated over glucuronidation in fetal lung, kidney, small intestine, and placenta whereas liver glucuronyltransferase exceeded β-glucuronidase at the later gestational periods. In adult guinea pigs, deglucuronidation exceeded glucuronidation in lung and small intestine whereas glucuronidation predominated in liver and kidney.

N-acetyltransferase is a supernatant enzyme and its perinatal development in rats and guinea pigs was studied using p-aminobenzoic acid as the substrate. Fetal and newborn enzyme activities were equivalent to adult levels at all gestational periods and newborn ages tested. Comparative activities were liver > placenta > lung at all ages. In a separate study, high N-acetyltransferase activities were detected in rabbit blastocysts and the significance of this finding is being investigated.

Comparative studies in adult tissues. Comparative N-acetyltransferase activities were liver > kidney > lung > small intestine in rats, liver > small intestine > lung > kidney in rabbits, and small intestine > kidney > liver > lung in guinea pigs. Sex differences in N-acetyltransferase from these species were small. Comparative data for PNP glucuronyltransferase were liver > kidney > testes > small intestine > lung > brain in rats, liver > small intestine > kidney > lung in rabbits, and liver > kidney > lung > small intestine in guinea pigs. These studies showed liver to consistently be the major glucuronidation site, although other tissues exhibited species variations. Guinea pig and rat testosterone glucuronyltransferase was found only in livers, but in rabbits steroid glucuronidation also occurred in small intestine microsomes.

Effects of 2,3,7,8-tetrachlorodibenzo-p-dixon (TCDD) pretreatment on glucuronyltransferase and other microsomal enzymes. Four days after a single oral dose of 5 μg TCDD/kg, liver cytochrome P-450, cytochrome b_5, biphenyl hydroxylation, benzpyrene hydroxylation, and PNP glucuronidation were markedly increased whereas testosterone glucuronidation and oxidative demthylations were not changed. Effects on hydroxylation and glucuronidation rates were observed at doses as low as 0.2 μg TCDD/kg and induction was still evident 73 days after treatment. Extrahepatic induction of PNP glucuronyltransferase and benzpyrene hydroxylation occurred in lung, kidney, small intestine, placenta, and brain whereas enzyme activities in the testes were unaffected.

Since TCDD is very teratogenic, we were interested in studying effects on fetal microsomal enzymes following maternal exposures. After pregnant rats were administered 3 μg TCDD/kg on the 5th, 10th, or 15th day of gestation, fetal liver glucuronyltransferase and benzpyrene hydroxylase activities were unaffected. However, after birth, marked increases over controls (3-10 fold) were observed. This induction could have been caused either by newborn exposure to TCDD via maternal milk or the activation of an inducing mechanism after birth. These possibilities were tested using foster mothers and results indicated that induction was caused by exposure to the mothers milk.

Pharmacokinetic studies with TCDD-^{14}C demonstrated that only small quantities of TCDD were detected in fetuses and the mothers milk contained high levels of unmetabolized TCDD. Excretion of TCDD in milk considerably lowered maternal body burdens. Low fetal TCDD levels indicates that either very little TCDD passes the placenta and/or very little is bound to fetal tissues. These studies also suggest that the teratogenic action of TCDD is possibly indirect since only small amounts of TCDD are detected or accumulate in the fetus.

Studies on the mechanism of action of TCDD on UDP glucuronyltransferase. Following 5 μg TCDD/kg pretreatment of rats, hepatic microsomal PNP glucuronyltransferase activity was elevated approximately 6-fold, whereas the hepatic glucuronyltransferase conjugating testosterone or estrone was unaffected. Solubilized and purified PNP glucuronyltransferase and steroid glucuronyltransferases from control and TCDD-treated rats exhibited the same relative activities (TCDD: control) as when the enzymes were bound to the endoplasmic reticulum. The effects of TCDD treatment on PNP glucuronyltransferase appeared to be related to increased amounts of liver enzyme for the following reasons: (1) K_m values for PNP and UDPGA were unchanged by TCDD treatments; (2) the magnitude of the TCDD-induced increase of PNP glucuronyltransferase activity was the same whether enzyme activity was measured in the presence or absence of Mg^{++} or Triton X-100; (3) TCDD, when added in vitro, had no detectable effect on enzyme activity; (4) TCDD treatment of rats did not change total hepatic microsomal phospholipid or cholesterol contents; (5) pH optima were unaffected by TCDD treatment; (6) solubilization of enzyme was not accompanied by a change in the TCDD induction effect; and (7) Actinomycin D appeared to block the initial phase of induction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The comparative rates of conjugation vs deconjugation pathways exert a strong influence on the toxicity and distribution of a number of foreign chemicals and many endogenous compounds including teratogens, carcinogens, mutagens, and other toxic compounds. Often these pathways are examined only in liver, although other organs such as kidney, lung, and small intestine are extremely important in the disposition of environmental chemicals. The comparative organ importance is, of course, strongly influenced by the route of entry and pharmacodynamic properties of the chemical.

Perinatal development studies of drug-metabolizing enzymes have in the past dealt primarily with oxidative systems. However, the conjugative systems are in many cases more important in fetal detoxication and steroid regulation pathways than are the mixed function oxidases. The few developmental studies that were done on conjugative systems were incomplete; dealing with only one enzyme in one tissue at one age and often using unsuitable incubation conditions. Our studies, and those done in collaboration with the Pharmacology Branch, will attempt to characterize the development of several conjugative systems in several different tissues at many different ages (fetal and newborn). These studies will yield information concerning: (1) which organ or organs are most important in conjugation at different ages; (2) the type of fetal conjugate that is likely to be formed at particular times during the gestational period; (3) how the fetus independently detoxifies foreign compounds; (4) the role of conjugation pathways in the regulation of many endogenous compounds,

and (5) prediction of synergistic or protective actions of one chemical to another in regards to fetotoxicity and teratogenesis.

PROPOSED COURSE: Future studies will be designed to: (1) discover the biochemical indicators that are associated with the development of N-acetyltransferase and glucuronyltransferase activity; (2) establish the influence of specific phospholipids to PNP and testosterone glucuronyltransferase activity in vitro and in vivo; (3) investigate the possible relationship between alterations in N-acetyltransferase activity and fetal CNS defects with special emphasis on serotonin N-acetylation; (4) characterize the biological significance of blastocyst N-acetyltransferase activity with emphasis on polyamine turnover; and (5) characterize the effect of TCDD on nuclear and cytoplasmic macromolecules in studies involving the cellular pharmacokinetics of TCDD-^{14}C.

PUBLICATIONS

Lucier, G. W., McDaniel, O. S., and Matthews, H. B.: Microsomal rat liver UDP glucuronyltransferase: effects of piperonyl butoxide and other factors on enzyme activity. Arch. Biochem. Biophys. 145: 520, 1971.

Lucier, G. W. and McDaniel, O. S.: Alterations in microsomal and lysosomal β-glucuronidase by compounds that induce hepatic drug metabolizing enzymes. Biochim. Biophys. Acta. 261: 168, 1972.

Lucier, G. W.: A comparative study on rat liver microsomal and lysosomal β-glucuronidase. Fed. Proc. 32: 2751, 1973.

Lucier, G. W., McDaniel, O. S., Hook, G. E. R., Fowler, B. A., Sonawane, B. R., and Faeder, E.: TCDD-induced changes in rat liver microsomal enzymes. Environ. Health Perspect. 5: 199, 1973.

Sonawane, B. R. and Lucier, G.W.: Hepatic and extrahepatic β-glucuronidase and glucuronyltransferase. Society of Toxicology, Washington, D. C., March 1974.

Lucier, G. W.: Nature of the enhancement of hepatic UDP glucuronyltransferase activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Fed. Proc. 1816, 1974.

Serial No.: NIEHS-ET-011
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Factors Affecting Hepatic Heme Synthesis During Mammalian Development"

PREVIOUS SERIAL NUMBER: NIEHS-PP-039

PRINCIPAL INVESTIGATOR: J. S. Woods, Ph.D.

OTHER INVESTIGATOR: V. V. Murthy, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	3.0
Professional:	1.5
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: (1) To elucidate the mechanisms governing the regulation of hepatic hematopoiesis during mammalian development through analysis of enzymes of the heme biosynthetic pathway, (2) to purify and biochemically characterize δ-aminolevulinic acid (ALA) synthetase from fetal rat liver and to compare its properties with those of the adult enzyme, (3) to study the effects of potentially porphyrogenic environmental agents on the regulation of hepatic ALA synthetase during mammalian development, (4) to determine the correlation between the biochemical abnormalities associated with porphyrin overproduction and the clinical symptoms of porphyria, and (5) to provide a biochemical and pharmacological basis for predictibility of human response to exposure to porphyrogenic environmental agents.

METHODS EMPLOYED: Standard published biochemical techniques are used to prepare subcellular fractions from mammalian tissues, to assay enzyme activities, and to determine porphyrin, heme precursors, and protein concentrations. Techniques employed for the partial purification of ALA synthetase from fetal rat liver mitochondria included solubilization of the membranous enzyme with the non-ionic detergent, Lubrol, followed by gel filtration on sephadex G-200, affinity chromatography, and sucrose density gradient centrifugation. Fetal animals of gestational age confirmed by date-breeding are used in developmental studies.

MAJOR FINDINGS: Studies were published on the role of heme in the regulation of ALA synthetase in fetal rat liver. These studies indicate that heme may facilitate the development of mitochondrial ALA synthetase, the first and rate-limiting enzyme in hepatic heme biosynthesis in adults, during the fetal hepatic development. In addition, they suggest that this enzyme may not become rate-limiting in hepatic heme biosynthesis until it becomes susceptible to repression by heme, a phenomenon which does not develop until near birth. In order to more clearly investigate the rate-limiting event in heme biosynthesis in fetal liver, studies are currently in progress to determine the pattern of ontogenic development of other major enzymes of the heme biosynthetic pathway. Thus far it appears that both ALA dehydratase and Uroporphyrinogen I Synthetase, the second and third enzymes in heme biosynthesis, are both significantly greater in activity than ALA synthetase throughout perinatal development and do not, therefore, play a rate-limiting role in this process. Whether heme synthetase, the final enzyme in the heme biosynthesis scheme, or glycine-N-acyltransferase, an enzyme which competes with ALA synthetase for the substrate, glycine, play a rate-limiting role is currently under investigation.

ALA synthetase from fetal rat liver mitochondria has been solubilized and purified approximately 25 to 30 fold. Procedures described in the literature for isolation of the enzyme from soluble and mitochondrial fractions of porphyric adult rat liver were not adaptable to purification of the fetal enzyme and required innovation as follows: solubilization of the enzyme from mitochondria using the non-ionic detergent, lubrol; gel filtration on sephadex G-200; and affinity chromatography using Bio-Rad affigel. Unlike the adult enzyme, fetal ALA synthetase is not inhibited by the end-product, heme, whereas both enzymes are strongly inhibited by high cation concentrations including NaCl and lead compounds. Fetal ALA synthetase has an absolute requirement for the substrates glycine and succinyl CoA, and the cofactor pyridoxal phosphate, with apparent Km values of 1×10^{-2}M, 1×10^{-4}M and 6×10^{-6}M, respectively. These studies suggest that elevated ALA synthetase activity in fetal mitochondria may partially be due to a lack of inhibition of this enzyme by heme. The high Km value for glycine suggests that the availability of this amino acid substrate may determine the rate-limiting event in fetal heme biosynthesis. Studies are currently in progress on glycine metabolism in fetal liver to assess this possibility.

Several environmental agents were investigated for potential porphyrogenicity in the mammal. 2,3,7,8-Tetracholorodibenzo-p-dioxin (TCDD), a potent inducer of ALA synthetase in chick embryo liver cells, was found to have surprisingly little or no porphyrogenic effects in mammals. These studies suggested the existence of an interesting species specificity in regard to the porphyrogenic effects of environmental agents, and studies are underway to examine the factors determining the differences in susceptibility to porphyrogenicity in mammals. Another chemical, methaqualone, is a quinazoline derivative with demonstrated therapeutic efficacy but is also a highly abused non-barbiturate hypnotic drug. Because methaqualone in known to interfere with microsomal drug metabolizing heme-containing enzyme systems, it was of interest to examine the possible porphyrogenic effects of this drug in mammals. These studies showed that methaqualone significantly stimulates the activity of ALA synthetase in adult rats. The mechanism of this increase was shown to be by induction of

enzyme synthesis. This drug is therefore contraindicated in the management of acute-intermittent porphyria in man. In addition, abuse of methaqualone might produce potentially dangerous alterations in hepatic heme metabolism.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The formation of non-erythropoietic heme is limited in mammalian liver cells by the activity of the enzyme, ALA synthetase. The availability of heme, as a key component of mitochondrial and microsomal cytochromes, directly influences the capacity of the organism to maintain normal metabolic processes, including the metabolism of drugs and other enviromental agents. The regulation of ALA synthetase during mammalian development is poorly understood. A clearer understanding of these processes, which are emerging from these studies, is required in order to predict and assess the effects on perinatal development of agents which alter the normal regulation of hematopoiesis in adults. It is anticipated that these studies will provide a basis for predictibility of human response to exposure to prophyrogenic environmental agents, and provide for the establishment of standards for protective and preventative measures to such human health hazards in the environment.

PROPOSED COURSE: Studies related to the regulation of hepatic hematopoiesis during mammalian development will continue. Particular emphasis will be placed on the investigation of the role of glycine metabolism and availability as a rate-limiting factor in heme biosynthesis in fetal liver. Further studies in the purification and characterization of ALA synthetase from fetal rat liver will be pursued with the aim of determining the factors affecting regulation of this enzyme during fetal hepatic development.

Studies to be initiated will attempt to draw a correlation between the biochemical abnormalities associated with porphyrin overproduction and the clinical symptoms of porphyria. The lack of any direct toxicity associated with increased blood and tissue levels of ALA, porphobilinogen, and other porphyrin precursors suggests that such increases may represent only one expression of a more widespread disturbance of cellular metabolism or organization. It is anticipated that studies on the regulation of heme biosynthesis in fetal liver may provide a clearer understanding of the abnormalities in cellular metabolism which characterize porphyria in the adult. Identification and classification of the abnormalities in cellular metabolism produced by prolonged exposure to low level doses of porphyrogenic environmental agents will be undertaken with the aim of providing a basis for the early predictivity of human responses to such exposure.

PUBLICATIONS

Woods, J. S.: Studies of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on mammalian hepatic δ-aminolevulinic acid synthetase. Environ. Health Perspect. 5: 221-225, 1973.

Woods, J. S.: Studies on the role of heme in the regulation of δ-aminolevulinic acid synthetase during fetal hepatic development. Molec. Pharmacol. In press.

Woods, J. S.: Studies on the pharmacology of methaqualone: induction of mammalian hepatic δ-aminolevulinic acid synthetase. Proc. Soc. Exptl. Biol. Med. In press.

Serial No.: NIEHS-ET-012
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Role of Superoxide Dismutase in Oxygen Tolerance and Oxygen Toxicity"

PREVIOUS SERIAL NUMBER: NIEHS-PP-035

PRINCIPAL INVESTIGATOR: J. D. Crapo, M.D.

OTHER INVESTIGATOR: P. E. Voytek, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To study the relationship between the level of superoxide dismutase (SOD) present in lung tissue and the ability of the animal to survive in pure oxygen or when given lethal doses of other pulmonary oxidants and (2) to determine the developmental characteristics of the enzyme SOD and to relate these to the changing ability of a developing animal to tolerate inhaled oxidants.

METHODS EMPLOYED: The aerotoxicology facilities at the Institute are used to expose test animals to various concentrations of oxygen and aerosols of superoxide dismutase. Routine biochemical, histological, immunological, and physiologic testing procedures are also used.

MAJOR FINDINGS:

(1) Pulmonary SOD and oxygen toxicity. Rats exposed to 85% oxygen for 7 days developed "tolerance" (the ability to survive in 100% oxygen) and at the same time the activity of pulmonary SOD increased 46% ($p<0.01$). The rate at which rats exposed to 85% oxygen acquired "tolerance" paralleled the time course for increased SOD activity. The rate at which "tolerance" was lost when these rats were returned to air correlated closely with the decline of SOD activity to normal levels. Guinea pigs, hamsters, and mice were also studied. When exposed to 85% oxygen, these species failed to demonstrate higher activity of pulmonary

SOD and also failed to acquire "tolerance". These findings are consistent with the postulate that the increased tolerance to hyperoxia observed in rats is due to protection by SOD from the toxic effects of O_2^-.

(2) Characteristics of rat SOD. Two structurally different forms of SOD exist--the mitochondrial and the cytosol forms. It has been shown that all organs examined in the rat contain SOD and that the electrophoretic mobility of the cytosol enzyme is identical in all organs. Rat liver SOD was isolated, purified, characterized, and specific activity determined.

(3) Antibody titrations of pulmonary SOD. The increase in lung SOD activity of oxygen-exposed rats could be due to increases in either one or both forms of the enzyme. Also, the change in SOD activity could be due to increased amounts of the enzyme or to a change in enzyme properties. To study these questions, rabbit antisera specific for rat cytosol SOD was prepared and antibody titrations were performed on lung homogenates using agarose gels stained for SOD with nitroblue tetrazolium. SOD/lung increased 42% ($p<0.01$) during hyperoxic exposure. Therefore, continuous exposure of rats to 85% oxygen appeared to increase absolute amounts of this enzyme rather than to alter enzyme properties. The entire increase in SOD determined by activity assays (46%) could be accounted for by the increased amounts of cytosol SOD. This suggests the possibility that hyperoxia leads to increased production of O_2^- primarily in the cytosol.

(4) Histological studies. The increased amounts of SOD produced during exposure to hyperoxia may be due to induction of the enzyme or to proliferation of a SOD rich cell type. Ultrastructural studies on mammals exposed to hyperoxia have shown proliferation of alveolar type II cells. The histology of rats exposed to 85% oxygen was studied by both light and electron microscopy. During the same time period that SOD increased and tolerance developed, there was an apparent increase in the relative numbers of alveolar type II cells. Further studies may demonstrate that increased numbers of alveolar type II cells during exposure to oxygen is related to SOD content or to the ability of type II cells to induce it.

(5) Developmental characteristics of SOD. Activity of SOD in lung and liver of rats from 14 days gestation to 13 weeks of age was determined. Lung SOD activity was found to be at normal adult levels at the 14th day of gestation and did not change with age. In the liver, a 2-3 fold increase in SOD activity was found during the last week of gestation and the first 2 weeks following birth.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The single electron reduction of oxygen, producing an extremely toxic free radical known as superoxide, has recently been shown to be a biologically important reaction. The enzyme SOD dismutes this free radical directly to O_2 and H_2O_2. It has been postulated that the presence of this enzyme protects against the toxic effects of oxygen and that it is necessary for survival in an oxygen-rich atmosphere. Oxygen is one of the most frequently used forms of medical therapy, and artificial atmospheres of oxygen are used in both industry and in medicine. All of the initial toxic effects of oxygen are manifest in the lung and deaths due to pulmonary oxygen toxicity are frequently reported. The current studies

represent one of the first attempts to propose a biochemical mechanism for pulmonary oxygen toxicity.

A large number of other environmental toxicants including ozone, NO_2, and paraquat may produce part of their toxic effects by way of the superoxide free radical, and the current studies with SOD and oxygen may have broad applications with regard to toxicity of environmental agents.

The developmental aspects of SOD should contribute to our understanding of the differing and changing abilities of neonatal animals to respond to pulmonary oxidants. Developmental abnormalities of SOD might be related to hyaline membrane disease.

PROPOSED COURSE: (1) The mitochondrial SOD will be purified from rat liver, characterized, and used to produce a rabbit antisera specific for it. (2) Further antibody titrations using both cytosol SOD antisera and mitochondrial SOD antisera will further characterize changes in SOD during O_2 exposure. (3) Fluorescent antibody techniques will be used to determine the histological location of SOD in rat lung and ferritin-tagged antibodies will be used to further determine the subcellular distribution of the SOD enzymes. (4) Rats will be exposed to aerosols of SOD to attempt to modify the toxic effects of oxygen and Cu-deficient rats will be used to evaluate their expected increased sensitivity to oxygen. (5) Other pulmonary oxidants will be evaluated for the development of cross tolerance after oxygen exposure and for their ability to increase SOD activity in the lung.

PUBLICATIONS

Crapo, J. and Tierney, D.: Superoxide dismutase and oxygen toxicity. Clinical Research 21: 222, 1973.

Crapo, J.: Superoxide dismutase in the development of oxygen tolerance. The Pharmacologist. 15: 239, 1973.

Crapo, J. and Tierney, D.: Superoxide dismutase and pulmonary oxygen toxicity. Am. J. Physiol. In press.

Serial No.: NIEHS-ET-013
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Studies of the Effects of Toxic Concentrations of Oxygen Upon Red Cell 2,3-diphosphoglycerate Metabolism In Rats"

PREVIOUS SERIAL NUMBER: NIEHS-PP-038

PRINCIPAL INVESTIGATOR: J. B. Weissberg, M.D.

OTHER INVESTIGATOR: J. D. Crapo, M.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	1.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: Studies of the role of red cell 2,3-diphosphoglycerate (2,3-DPG) in oxygen toxicity to rats were undertaken in order: (1) to further understanding of the basic pathophysiology of oxygen toxicity with attention to the hitherto unexplored role of the red cell in this process; (2) to investigate the possibility that red cell 2,3-DPG metabolism constitutes an important compensatory mechanism to account for tolerance to toxic levels of oxygen; (3) to investigate the more general question of how 2,3-DPG metabolism responds to elevations in arterial oxygen tension; (4) to assess possible untoward effects of oxygen therapy consequent upon alterations in 2,3-DPG metabolism; and (5) to contribute to the present limited understanding of the mechanisms whereby 2,3-DPG levels in red cells are regulated.

METHODS EMPLOYED: Charles River male rats between 4 and 12 weeks of age (with weights between 80 and 350 grams) were used in these studies. Oxygen exposures were carried out at 23°C in polystyrene chambers. The concentration of oxygen was controlled by mixing pure oxygen from a liquid oxygen tank with air using air flow meters. Flow rates sufficient to provide 5-7 air changes per hour were used. Oxygen concentrations were determined in a blood gas analyzer. Blood for 2,3-DPG determinations was withdrawn from the aortas of rats anesthetized with sodium pentobarbital. Red cell content of 2,3-DPG was measured by means of a colorimetric assay based on the reaction of molybdenum with inorganic phosphorus hydrolyzed from 2,3-DPG in the presence of phosphoglycerate mutase and phosphoglycolic acid.

MAJOR FINDINGS: A differential response in terms of survival in 100% oxygen was observed between rats 4 and 12 weeks of age. The older rats died after 66 + 6 hours of exposure, while the younger animals survived much longer. No change in red cell 2,3-DPG levels of 12 week-old rats was observed after 24, 48, and 60 hours of exposure to 100% oxygen. In contrast, 4 week-old rats showed a significant ($p<0.001$) decline in 2,3-DPG after 24 hours exposure to 100% oxygen. This effect was greatest during 24-36 hours but persisted throughout a 7-day exposure period. Concentrations of 40, 60, and 80% oxygen similarly lowered the red cell 2,3-DPG content in 4 week-old rats. A return of 2,3-DPG levels to normal values was observed 6 hours after animals were returned to atmospheric oxygen tension.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 2,3-DPG is known to decrease the affinity of hemoglobin for oxygen by selectively binding to deoxyhemoglobin. Elevations in red cell 2,3-DPG with resultant enhanced availability of oxygen to tissues have been reported in instances of lowered arterial oxygen tension. The present studies are concerned with the response of 2,3-DPG to increased arterial oxygen tension. Oxygen toxicity is a clinical entity that is widely encountered and poorly understood. A study to advance understanding of the pathophysiologic mechanism involved is essential to the eventual discovery of needed prophylactic and therapeutic measures. A decline in 2,3-DPG levels after exposure to high oxygen tensions might decrease oxygen availability to tissues and thus protect against oxygen's toxicity. If red cell 2,3-DPG has a role in toxicity and tolerance, the therapeutic implications are significant. Of equal significance would be the elucidation of physiological events that are observable at the level of the red cell not only in oxygen toxicity but also in the normal course of oxygen therapy. Lowered 2,3-DPG levels with resultant decreased availability of oxygen to tissues would be disadvantageous in the interval between withdrawal of oxygen at high tensions and return of red cell 2,3-DPG to normal levels.

PROPOSED COURSE: To establish that the observed lowering of red cell 2,3-DPG does result in decreased availability of oxygen to tissues, it remains to perform oxygen-hemoglobin dissociation studies and to measure tissue-oxygen uptake. These studies will be carried out in 4 and 12 week-old rats before and after oxygen exposure. Investigation of the mechanism for the observed change in red cell content of 2,3-DPG is also planned. This will include study of the response of 2,3-DPG mutase and 2,3-DPG phosphatase to increased oxygen tension both *in vivo* and *in vitro*.

PUBLICATION

Weissberg, J. B., and Crapo, J. D.: Red cell 2,3-diphosphoglycerate and oxygen toxicity in rats. Annual Meeting of the Society of Toxicology, March 1974.

Serial No.: NIEHS-ET-014
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "DNA Repair in Mouse Oocytes and Zygotes"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: P. E. Voytek, Ph.D. and K. Y.-V. Ku, Ph.D.

OTHER INVESTIGATORS: L. A. Moustafa, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	1.4
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVE: (1) To establish direct evidence for DNA repair mechanisms in primary mouse oocytes and zygotes and (2) to assess the effects of physical and chemical agents known to be cytotoxic to mammalian cells on the repair process.

METHODS EMPLOYED: The following is a generalization of the procedure used (controls to determine optimal concentrations and conditions are not included): (1) Primary oocytes from CD1 Swiss albino mice were obtained by treating females with pregnant mare serum (PMS) followed 46 hours later by administrating human chorionic gonadotropin (HCG). After 8 to 10 hours, the animals were sacrificed and primary oocytes removed. To obtain fertilized eggs at the single cell stage female mice were initially treated as above but after HCG treatment, the females were exposed to males for 10 to 12 hours. Eggs were removed from those animals containing vaginal plugs. Yields were in the range of 30 eggs per mouse. (2) Cell types were incubated in arrested growth medium containing hydroxyurea and chemical agents (i.e., diethylstilbestrol and possibly some organochlorine insecticides) to be tested followed by a second incubation in a medium containing hydroxyurea and tritiated thymidine. (3) Cells were incubated in arrested growth medium with hydroxyurea, followed by irradiation (ultraviolet or X radiation) and proceeded by incubation in hydroxyurea and ^{3}H-Thymidine. (4) Repair was assessed by autoradiography. (5) And, treated eggs were artifically fertilized and reimplanted.

MAJOR FINDINGS: The findings were that UV light induced DNA synthesis does occur in primary mouse oocytes by autoradiographic studies of tritiated thymidine incorporation into DNA. After injury by UV light, an increase in grain counts was only observed along the chromosomes and although oocytes contain much cytoplasmic DNA, no increase in nonnuclear grain counts was observed above controls. It has been previously shown that adult mouse sperm and adult human sperm do not undergo UV light induced DNA synthesis as judged by tritiated thymidine incorporation into DNA. Our preliminary findings indicated no detectable thymidine kinase activity in adult mouse sperm cell. However, *in vitro* studies indicated the existence of a DNA polymerase activity. This poses an important question as to whether repair occurs in adult sperm but cannot be detected by thymidine incorporation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Man today is exposed to an increasing number of chemical and physical hazards which can result in genetic damage which in turn could lead to cellular mutations, transformation, carcinogenesis, decrease in survival time, and death. Therefore, acquiring knowledge of genetic repair mechanisms capable of correcting such damage is extremely important. However, little effort has been expended on the repair processes in germ cells, and, in particular, oocytes and fertilized eggs. The importance of DNA repair during early cell stages of development may be greatly magnified over repair found in somatic cells because of the many time sequences in events that may be gene regulated. It would seem that damaged genetic material would have to be rapidly repaired or the development of the embryo would cease or would proceed abnormally. Therefore, the influence of many potential toxic agents, either physical or chemical, on the repair processes during early developmental stages could present hazardous consequences.

PROPOSED COURSE: Continue study of DNA repair processes in primary oocytes and zygotes and examination of the influence of potential toxic agents on repair in these cells.

Serial No.: NIEHS-ET-015
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Investigation of Thymidine Kinase Enzymes in Independently Derived Mutant Lines of L5178Y Mouse Lymphoma Cells"

PREVIOUS SERIAL NUMBER: NIEHS-PP-037

PRINCIPAL INVESTIGATOR: P. E. Voytek, Ph.D.

OTHER INVESTIGATOR: D. Clive, Ph.D., Environmental Mutagenesis Branch

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.5
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate whether thymidine kinase activity in the various thymidine kinase mutant cells is caused by mutations in control or structural genes; and (2) if structural changes are evident, to carry out studies of the physical and kinetic parameters of the enzymes.

METHODS EMPLOYED: Production and isolation of various mutant stock cells were obtained as described in Individual Project Reports, Serial No. NIEHS-M-004 and NIEHS-M-006, July 1, 1971 through June 30, 1972. Purification techniques were carried out to enable characterization of the different mutated thymidine kinases. Electrophoretic techniques, density gradient centrifugation, and examination of kinetic parameters were used to investigate enzymatic differences. Thymidine kinase from the original lymphoma cell line which contains the diploid locus was investigated first to assess the characteristics of these two (possibly identical) gene products.

MAJOR FINDINGS: Distribution studies of thymidine kinase in wild type cells and two mutant cell types were carried out by measuring enzymic activities in the 100,000 x g supernatant liquids and their resuspended pellets after sonication. No activity was found in the supernatant from wild type cells, approximately 10% of the enzymic activity could be extracted from the first mutants studied, whereas 100% of the total thymidine kinase activity was located in the supernatant fluid from the second mutant cells examined. However, when sonications were performed in the presence of 3M LiCl, 50% of the

thymidine kinase activity from wild type cells was extractable into the 100,000 x g supernatant fluid. LiCl did not change the distribution of activity for the first mutant cell types. Kinetic studies in which thymidine was the variable substrate showed that apparent Km values were not significantly different for sonicate, 100,000 x g supernatants or resuspended pellet enzyme preparations within each cell type, but Km values for both mutant cell types were approximately four times greater than wild type cell preparations. When ATP-Mg was the variable substrate, high concentrations inhibited thymidine kinase activity in wild type and first mutant cell preparations, but no inhibition was noticed for the second mutant cell enzyme preparations.

Distribution and kinetic differences among the wild type cells and both mutant cell lines provide evidence that structural rather than regulatory mutations have occurred.

PROPOSED COURSE: Because of the solubility problems encountered with wild type and mutant two preparations of thymidine kinases, mutagenic differences among the cell enzymes at the structural level would be different. An enzyme system where both wild type cells and mutant are all soluble would be a more ideal system. The possibilities for obtaining such a system are presently being explored. A manuscript describing our findings with L5178Y mouse lymphoma cells is in preparation.

Serial No.: NIEHS-ET-016
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "In Vitro Culturing of Mammalian Gonadal Tissues"

PREVIOUS SERIAL NUMBER: NIEHS-PP-034

PRINCIPAL INVESTIGATORS: J. W. Spalding, Ph.D., and J. A. McLachlan, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNIT: None

MAN YEARS

Total:	1.6
Professional:	0.8
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVE: To develop a viable tissue or organ culture system of mammalian gonads for the purpose of investigating the mechanism by which chemical agents affect oogenesis and spermatogenesis.

METHODS EMPLOYED: Fetal gonads from C 57 Bl/6 mouse embryos were removed at intervals from 12 through 17 days gestation time and explanted as organ cultures in vitro. Tissue culture medium supplemented with fetal calf serum was used to support the growth of the explants. Growth and differentiation of several different cell types was followed by morphological and histochemical parameters.

MAJOR FINDINGS: Oocyte maturation in ovary explants taken from embryos at 16 to 17 days gestation time was followed for 12-14 days in culture. Oocyte maturation in vitro closely paralleled oocyte maturation in vivo up to 25 days post-conception time. The oocytes enlarged; formation of primordial follicles occurred; and, in some instances, follicle maturation proceeded to the point where bilaminar and trilaminar follicles could be observed. Follicle maturation did not proceed beyond the trilaminar stage even though the tissue retained metabolic viability in culture. By 12-14 days in culture, few, if any, small primordial follicles remained in the explant.

Twelve day fetal ovaries were grown in organ culture in order to observe oogenesis in vitro. Growth and maturation of the 12 day explants paralleled growth in vivo up to 6-7 days in culture. At this time, the oocytes rapidly

degenerated, and the ovary usually became devoid of decernable oocytes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Various environmental agents, including drugs, are known to effect reproduction and development. The specific molecular effect of these agents on germ cells (oocytes and spermatocytes) during the early stages of development are relatively unknown. It can be expected that defective germ cells may result in offspring which are sterile or have a wide range of inborn genetic deficiencies. It would be very beneficial to have an in vitro model of embryonic and postnatal germ cell differentiation. In vitro studies could then be correlated with ongoing studies of the in vivo effects of chemicals on gametogenesis and fertility. Hopefully, a fast in vitro test system can be developed to predict in vivo effects of chemicals. In addition, this in vitro system would provide an opportunity to further study mechanisms which regulate differentiation and maturation.

PROPOSED COURSE: In vitro studies with the 16 day fetal ovaries (post-conception time) will be directed toward obtaining consistent and reproducible stages of growth and maturation in order to develop parameters that may be used to examine the effect of selected xenobiotics on oocyte growth and maturation.

Studies will be continued with 12 day fetal ovaries in order to develop cultural conditions that will permit oocytes to remain viable and to continue growth and maturation beyond 7 days in organ culture.

Twelve day and 16 day fetal mouse testes will be studied under cultural conditions similar to those described above. The purpose will be to determine the degree of growth and differentiation that can occur in fetal mouse testicular tissues grown in vitro.

Serial No.: NIEHS-ET-017
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Mode of Toxicity of Organochlorine Xenobiotics in Cultured Mouse Lymphoma Cells"

PREVIOUS SERIAL NUMBER: NIEHS-PP-028

PRINCIPAL INVESITGATOR: J. W. Spalding, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.4
Professional:	0.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To study the nature of the interaction of organochlorine compounds with the plasma membrane of mammalian cells grown in vitro, and to determine how this interaction relates to the toxic properties of these same organochlorine compounds.

METHODS EMPLOYED: The effect of the interaction of organochlorine compounds with the plasma membrane of mouse L5178Y lymphoma cells is observed in susceptible and resistant lymphoma cell sublines. The parameters studied are: (1) the transport into cells and subsequent incorporation of appropriate precursors into DNA, RNA, protein, and phospholipids and (2) the degree of binding which occurs between the organochlorine compounds and the cell plasma membrane.

MAJOR FINDINGS: Representatives of several different classes of organochlorine compounds, e.g., DDT, chlordane and 4-chlorobiphenyl (4-Cl-BP), are toxic to L5178Y cells. 4-Chlorobiphenyl inhibits the rate of transport of selected small molecular weight precursors of protein and nucleic acid syntheses. All three above named organochlorine compounds stimulate the incorporation of choline into phosphatidylcholine but have little or no effect on the rate of choline transport across the cell membrane. It has been shown that DDT and 4-Cl-BP are absorbed on the cell membrane but do not penetrate into the cell. Thus, the stimulation on phosphatidylcholine metabolism by these compounds is effected without the agents being incorporated into the cell.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mode of toxicity exhibited by the organochlorine pesticides and other related xenobiotics, e.g., PCB's, is still unknown at this time. Tissue culture cell systems provide useful models whereby the molecular mechanism of toxicity expressed by these compounds may be investigated. The studies described may lead to an understanding of how DDT, chlordane, PCB's, and other highly water-insoluble and persistent organochlorine compounds effect their toxicity in biological systems.

The results of the investigations described above suggest that the organochlorine compounds express their toxicity by altering the plasma membrane in such a way that the rate of transport of essential small precursor molecules into cells (tissues) is effected. In addition, they alter the metabolism of phospholipids and thus, can effect the integrity of the various membrane structures of cells (tissues).

PROPOSED COURSE: The studies on phospholipid metabolism will be continued on a limited basis.

PUBLICATIONS

Spalding, J. W., Ford, E., Lane, D., and Blois, M.: The character of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane resistance in mouse L5178Y Lymphoma cells. Biochem. Pharmac. 20: 3185-3196, 1971.

Serial No.: NIEHS-ET-018
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Morphologic Effects of Heavy Metals"

PREVIOUS SERIAL NUMBER: NIEHS-PP-036

PRINCIPAL INVESTIGATOR: B. A. Fowler, Ph.D.

OTHER INVESTIGATORS: R. A. Goyer, M.D., Department of Pathology, University of North Carolina at Chapel Hill
H. S. Jones, Ph.D., Officer of the Director
K. R. Mahaffey, Ph.D., F.D.A., Washington, D.C.
G. W. Lucier, Ph.D., Environmental Toxicology Branch
G. E. R. Hook, Ph.D., Pharmacology Branch
M. R. Krigman, M.D., Department of Pathology, University of North Carolina at Chapel Hill
P. Mushak, Ph.D., Department of Pathology, University of North Carolina at Chapel Hill
D. A. Wolfe, Ph.D., Atlantic Estuarine Center, National Marine Fisheries Service, Beaufort, N.C.
G. T. Barthalmus, Ph.D., Department of Zoology, North Carolina State University at Raleigh
N. A. Mercando, M.S., Department of Zoology, North Carolina State University at Raleigh

COOPERATING UNITS: Department of Pathology, University of North Carolina at Chapel Hill
F.D.A., Washington, D.C.
Pharmacology Branch
National Marine Fisheries Service, Beaufort, N.C.
Department of Zoology, North Carolina State University at Raleigh

MAN YEARS

Total:	3.6
Professional:	2.6
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: (1) To define the ultrastructural effects of heavy metals and other environmental toxicants on mammalian and marine invertebrate tissues, and (2) to correlate these observations with tissue levels and biochemical alterations in blood and viscera.

METHODS EMPLOYED: Routine chemical, histologic and electron microscopic methods were used. Special emphasis was placed on the determination of tissue levels of heavy metals and subcellular morphologic changes. Behavioral tests were used to evaluate the sublethal effects of heavy metals on marine crustaceans.

MAJOR FINDINGS:

(1) Arsenic. Studies on the differential uptake by male and female rats of arsenite (As^{+3}) and arsenate (As^{+5}) administered at levels of 3, 30, and 90 parts per million (ppm) in the drinking water for 14 weeks showed that in general total arsenic levels in the kidneys, liver, gonads, and fur were highest when As^{+3} was administered rather than As^{+5}. Male rats were found to have higher renal levels of total arsenic than females for either As^{+3} or As^{+5} while females had higher liver levels for either form. Ultrastructural studies on kidneys from these animals disclosed mitochondrial swelling at the higher dose levels for either As^{+3} or As^{+5}.

(2) Cadmium. Cadmium given in the drinking water at concentrations of 0.2, 2, 20, or 200 ppm to rats on low or normal calcium diets caused marked cellular hypertrophy, increased amounts of rough endoplasmic reticulum (RER), cellular interdigitation, and formation of electron dense cytosomes in parathyroid gland cells. The dense cytosomes were found to contain iron by X-ray microanalysis. Morphometric studies on renal arteries from these same animals disclosed constriction of the smaller arteries at 6 weeks but not 12 weeks. Glomerular hypertrophy and interstitial fibrosis were also observed. Studies on clams and oysters placed in sea water containing cadmium at concentrations of 0.1, 1, or 10 ppm showed that half of the high dose oysters were dead at 3 days while half of the high dose clams did not die until the 7th day. The most readily apparent histologic lesion in these animals was necrosis of the columnar epithelial cells lining the gut.

(3) Methylmercury. Studies were conducted on the lysosomal uptake of mercury in kidneys of rats given access to drinking water containing methylmercury at concentrations of 3, 5, or 10 ppm for 4 weeks which showed mercury accumulation in lysosomes as demonstrated by X-ray microanalysis. Further work on phenobarbital protection against methylmercury nephrotoxicity demonstrated decreased creatinine clearances in rats given 5 ppm methylmercury in their drinking water for 4 weeks but not those so treated and given weekly injections of phenobarbital.

(4) Inorganic Mercury (Hg^{++}). Studies concerning the toxicity of Hg^{++} to clams and oysters placed in sea water at concentrations of 0.1, 1, or 10 ppm mercury disclosed that mercury is more toxic than cadmium to these animals. All of the high dose oysters and about half of 1 ppm dose group oysters were dead after 3 days of exposure. Half of the clams placed in the 10 ppm mercury solution were dead at 5 days instead of 7 as with cadmium. Necrosis of the columnar epithelial cells lining the gut was the most readily apparent histologic change. Electron microscopy of mantle epithelial cells of the clams demonstrated a dose-related increase in occurrence of electron dense cytosomes within the apical cytoplasm. The cytosomes were found to contain large amounts of iron and some mercury by X-ray microanalysis. Mercuric ion in sea water at

concentrations of .01, .05, and .1 ppm decreased the ability of grass shrimp and hermit crabs to respond to behavioral stimuli although the animals fed and appeared physiologically normal.

Dioxin. Administration of a single oral dose of 2,3,7,8 tetrochlorodibenzo-p-dioxin at a level of 25 µg/kg to rats resulted in a massive increase in renal microsomal enzyme activity with associated increases in smooth endoplasmic reticulum (SER) that were largely confined to proximal tubule cells of the S_3 (Pars recta) segments. These changes were found to persist for up to 16 days after treatment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to delineate ultrastructural criteria for evaluating the toxicity of environmental agents administered at low levels. These findings are associated with specific biochemical events and tissue concentrations of these agents. Because the tissue requirements for electron microscopy are small, it might eventually be possible to evaluate the toxicity of environmental agents, e.g., heavy metals, through the use of biopsy procedures. This is particularly true of the X-ray microanalysis technique which allows localization of heavy metals within cells. The studies of marine organisms are of value because they allow the investigator to compare the toxic effects of heavy metals on mammalian systems with those observed in less complicated marine animals. A second point is that man is commonly exposed to heavy metals through consumption of shellfish. A knowledge of where and how these metals are localized within these organisms is of great potential value.

PROPOSED COURSE: The effects of heavy metals on cellular ultrastructure will be continued. In addition, the influence of one heavy metal on the toxicity of another will also be investigated. Further studies are anticipated in order to compare observed cellular alterations of heavy metals injected into tissues with those seen when the metals are given in drinking water or added to food. More extensive comparative marine studies are also anticipated.

PUBLICATIONS

Fowler, B. A. and Jones, H. S.: Morphologic alterations of renal blood vessels after chronic cadmium exposure. The Pharmacologist. 15: 227, 1973.

Goyer, R. A., Brown, M. M., Rhyne, B. C., and Fowler, B. A.: Dose-response of renal mitochondria to oral ingestion of arsenate (Na_2H as $O_4 \cdot 7H_2O$) in the rat. American Journal of Pathology 74: 57a, 1974.

Fowler, B. A.: Lysosomal uptake of mercury in renal proximal tubule cells of rats given methyl mercury. Fed. Proc. 33: 309, 1974.

Jones, H. S. and Fowler, B. A.: Morphologic appearance of the parathyroid glands after chronic cadmium exposure. Fed. Proc. 33: 216, 1974.

Fowler, B. A., Lucier, G. W., Brown, H., and McDaniel, O.: Ultrastructural changes in rat liver cells following a single oral dose of TCDD. Environ. Health Perspect. 5: 141-148, 1973.

Lucier, G. W., McDaniel, O. S., Fowler, B. A., Faeder, E., Hook, G., and Sonawane, B.: Studies on TCDD-induced changes in rat liver microsomal and mitochondrial enzymes. Environ. Health Perspect. 5: 199-209, 1973.

Fowler, B. A. and Parker, P.: Observations on the preparation of epoxy embedded tissue samples for energy-dispersive X-ray analysis. Stain Technol. 48: 333-335, 1973.

Fowler, B. A.: The morphologic effects of mercury, cadmium, lead, and arsenic. Proceedings: Sixteenth Water Quality Conference. Trace Metals in Water Supplies: Occurrence Significance and Control. In press.

Fowler, B. A., Brown, H. W., Lucier, G. W., and Beard, M. E.: Mercury sequestration in renal lysosomes of rats ingesting methyl mercury: Ultrastructural observation and energy dispersive X-ray analysis. Archives of Pathology. In press.

Serial No.: NIEHS-ET-019
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Purification of the Beef Brain ($Na^+ + K^+$) ATPase System"

PREVIOUS SERIAL NUMBER: NIEHS-ASC-040 and NIEHS-PP-048

PRINCIPAL INVESTIGATOR: C. W. Sharp, Ph.D.

OTHER INVESTIGATOR: W. E. Wilson, Ph.D.

COOPERATING UNITS: None

MAN YEARS

Total:	1.0
Professional:	1.0
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVE: To examine several partially purified $Na^+ + K^+$ ATPase preparations in an effort to further evaluate this system as a suitable membrane model for the analysis of the interactions of hydrophobic and other environmental agents with biological systems. The primary purpose of this study was to analyze the role of phospholipids in these interactions. This was accomplished through pertubation, extraction, and reconstitution with various specific phospholipids.

METHODS EMPLOYED: Conventional enzyme techniques were utilized to study the rates of hydrolysis of various phospholipids and to monitor their involvement in the protection of the $Na^+ + K^+$ ATPase. Also liquid and thin layer chromatography, acrylamide electrophoresis, density gradient and differential ultracentrifugation, spectrophotometry, recording pH stat analysis, radioactive tracer techniques, and ultrasonic solubilization were used.

MAJOR FINDINGS: The selective protection of phosphatidyl serine (PS) against inactivation of the $Na^+ + K^+$ ATPase of cow brain, rabbit kidney. and eel was investigated. Inactivation by phospholipase was used as a model. The rates of substrate hydrolysis by a purified preparation of V. russelli phospholipase A in decreasing order are as follows: ATPase phospholipid (membrane-bound), detergent solubilized phosphatidyl choline (PC), solubilized phosphatidyl inositol (PI), PS, PI, and PC. Neither PC nor PS altered the rate of fatty acid release by this phospholipase A in the presence of the ATPase. Another phospholipase A purified from N. naja venom also hydrolysed the membrane

bound phospholipids more rapidly than the detergent solubilized PC, but hydrolyzed PC and PS most slowly in aqueous media. However, inactivation of the $Na^+ + K^+$ ATPase by either phospholipase A was selectively protected against or reversed by PS. Also, only PS protected against inactivation by these phospholipases in the presence of DOC. The same concentration of DOC markedly activated the hydrolysis of PC, whereas the hydrolysis of PS was little changed. These results indicate that the protection protocol may represent a valid approach for evaluating the specificity of phospholipid interaction in the stabilization of membranal enzyme systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Suitable systems which are readily available, stable, and easily reproduced are not available for demonstrating the interactions of various environmental compounds with membranal systems. These results lead to a further understanding and utilization of a basic biological system known to be affected by various chlorinated hydrocarbons and which may be a suitable model for evaluating the effects of other compounds.

PROPOSED COURSE: This concludes the work on this project.

Serial No.: NIEHS-ET-020
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "An Investigation of the Molecular Bases for Modulations in Plasma Membranal ($Na^+ + K^+$) ATPase Behavior in Response to Chlorinated Hydrocarbon Inhibitors, Detergents, Phospholipids, Drugs, and Hormones"

PREVIOUS SERIAL NUMBER: NIEHS-PP-047

PRINCIPAL INVESTIGATOR: W. E. Wilson, Ph.D.

OTHER INVESTIGATOR: C. W. Sharp, Ph.D.

COOPERATING UNIT: None

MAN YEARS

Total	0.75
Professional:	0.75
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVE: In order to develop techniques which would permit easier detection of selectivity among phospholipids for stabilizing membranal ($Na^+ + K^+$) ATPases (see publication #2), it was determined that the test involving selective phospholipid protection against ($Na^+ + K^+$) ATPase inactivation by phospholipase A should be investigated further.

METHODS EMPLOYED: Customary enzyme purification and assay techniques were used. Enzyme assays required the use of a pH stat.

MAJOR FINDINGS: Phospholipase A from venoms of Russels viper and from Naja naja cobra were partially purified. Substrate specificities of phospholipases were determined in the presence and absence of various detergents. Selectivity among phospholipids for protection against ($Na^+ + K^+$) ATPase inactivation by phospholipases A has been found to be very similar to that for reversal of ($Na^+ + K^+$) ATPase inactivation following exposure to phospholipase A.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study represents the final aspect of our investigation of the phenomena involved in inactivation of ($Na^+ + K^+$) ATPases by chlorinated hydrocarbons. This study is the only definitive biological study at the molecular level which addresses itself to rationalization of the mode of action of chlorinated

hydrocarbon inactivation of a toxicologically significant enzyme system. This study also demonstrates that the influence of phospholipases A and C on (Na^+ + K^+) ATPase behavior may be mimicked by some perturbation agents (i.e., by certain anionic amphiphilic compounds or certain chlorinated hydrocarbons).

PROPOSED COURSE: This project has been completed.

PUBLICATIONS

Wilson, W. E. and Sharp, C. W.: Phospholipid selectivity in stabilization of several mammalian (Na^+ + K^+) ATPases. 9th International Congress of Biochemistry, Stockholm. Abstract 5d 13, 1973.

Sharp, C. W., Hunt, D. G., Clements, S. T., and Wilson, W. E.: The influence of dichlorodiphenyltrichlorethane, polycholorinated biphenyls and anionic amphiphilic compounds on stabilization of sodium and potassium-activated adenosine triphosphatase by acidic phospholipids, Mol. Pharmacol. 10, 119, 1974.

Sharp, C. W. and Wilson, W. E.: Nature of the acidic phospholipid protection against phospholipase A inactivation of (Na^+ + K^+) ATPase. Fed. Proc. 34 (2), 1974.

Serial No.: NIEHS-ET-021
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "$(Na^+ + K^+)$ATPase Behavior and Plasma Membranal Integrity in Tissues of Developing Animals"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: W. E. Wilson, Ph.D.

OTHER INVESTIGATORS: C. W. Sharp, Ph.D., N. Chernoff, Ph.D., and P. W. Albro, Ph.D.

COOPERATING UNITS: Toxic Effects Branch, EPA, and Environmental Biology and Chemistry Branch

MAN YEARS

Total:	2.25
Professional:	1.25
Other:	1.00

PROJECT DESCRIPTION

OBJECTIVES: (1) To ascertain whether and to what extent brain plasma membranal properties of fetal tissue resemble those from the adult; (2) to ascertain the extent to which alterations in plasm membranal structure and function may correlate with fetal-toxic and/or teratogenic responsiveness. Secondary objectives: (3) to ascertain threshold conditions which would accentuate teratogenic responsiveness to pantothenic acid or essential fatty acid deficiencies; and (4) to determine whether teratogenic responsiveness to a dietary deficiency corresponded to that resulting from substitution of vitamin analogs in the diet.

A broad spectrum of teratogenic responsivess has been observed among fetuses of rats exposed to diets deficient in pantothenic acid or essential fatty acids (M. M. Nelson, et. al., Nutrition 62: 395-405 1957; A. Giroud, Fed. Proc. 27: 163-184, 1968; G. T. Grabowski, Adv. Teratology 4: 126-167, 1970). Pantothenic acid influences the conversion of linoleate to arachidonate (A. Fidanza, Acta Vitaminol Enzymol 25: 135-144, 1971). Thus, we have (a) investigated the influence of dietary deficiencies in pantothenic acid and essential fatty acids on teratogenic responses in the rat fetus and (b) attempted to ascertain the influence of diet on lipid structure and enzyme function in plasma membranes.

METHODS EMPLOYED: Membranal ($Na^+ + K^+$)ATPases were prepared from maternal and fetal brain and from placental tissue. The brain enzymes were partially characterized according to conventional types of techniques. Plasma electrolyte compositions were determined using flame photometry. Phospholipid files were determined using thin layer chromatography coupled with phosphate analysis. Fatty acid compositions of phospholipids were determined using gas liquid chromatography. Tissues were obtained from animals fed defined diets.

MAJOR FINDINGS: We were able to obtain reasonably satisfactory ($Na^+ + K^+$) ATPase preparations from fetal and adult brain but not from placental tissue. Partial kinetic characterization of the rat brain ($Na^+ + K^+$)ATPases indicated that the K_m values for Na^+ and K^+ were similar, while that for ATP appeared different, in fetal versus adult enzymes. Both fetal and adult ($Na^+ + K^+$) ATPases appeared to be selectively stabilized by acidic phospholipids.

While plasma concentrations of Na^+ are essentially identical in fetal and adult animals, fetal plasma K^+ concentrations normally ranged from about 75% to 200% higher than those of the maternal animals.

Pregnant rats on a pantothenic acid deficient diet yielded 20 day-old fetuses which showed no gross morphological abnormalities. Pregnant rats on a diet deficient in pantothenic acid and essential fatty acids yielded fetuses with a significant number of gross fetal abnormalities and fetal resorptions.

Profiles of total phospholipids and of fatty acids from the phospholipids were determined. Diet affected the fatty acid composition of placental phospholipids, but did not affect the profile of phospholipids in that tissue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ($Na^+ + K^+$)ATPases in plasma membranes are hormone-responsive components of plasma membranes which function as regulators of alkali metal ion distribution and of plasma membranal electrical potential gradients. Since mammalian ($Na^+ + K^+$)ATPases seem to be selectively stabilized by acidic phospholipids, this stabilization phenomenon may be utilized to provide a reflection of structural and functional integrity of brain plasma membranes. Phenomena which result in improper phospholipid biosynthesis could conceivably alter membranal ($Na^+ + K^+$) ATPase activity resulting in altered osmoregulatory control, altered electrolyte distributions and, possibly, intracellular pH changes and/or edema. Our efforts will facilitate acquisition of new knowledge about nerve membranal structure and function during fetal and neonatal development. From an investigation of the possibility of alteration of brain plasma membranal structure and function by teratogenic analogs (dietary deficiencies), we may eventually obtain some insight into the possibilities of the influence of environmental factors including diet composition on such phenomena as behavior, learning disabilities, etc., as well as upon the types of physiological responsiveness mentioned above.

PROPOSED COURSE: We shall continue investigation of the influence of alterations in the maternal diet on the integrity of brain plasma membrane structure and function in the developing rat. This effort will also involve examination of the influence of dietary alterations on plasma electrolyte composition.

The dietary variations will initially involve (a) deletion of pantothenic acid and/or addition of pantothenic acid analogs and (b) alteration of dietary content of essential fatty acids. Subsequently, we plan to investigate the influence of added metals, drugs, and/or other teratogenic compounds on responsiveness to diets contain approximately threshold levels of essential fatty acids or vitamins.

Serial No.: NIEHS-ET-022
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants"

PREVIOUS SERIAL NUMBER: NIEHS-PP-004

PRINCIPAL INVESTIGATOR: W. Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: M. E. Porter, P. M. Hudson, B.S., C. W. Boyd, and G. L. Stranzinger, Ph.D.
J. Zinkl, Ph.D., and J. E. Thigpen, Ph.D., Environmental Biology and Chemistry Branch
W. Wood, Ph.D., C. Dawe, M.D., M. Chirigos, Ph.D., and J. Rice, Ph.D., NCI
A. Moore, P. Mortillaro, J. Romine and A. Godwin, MAPB
R. Martensen, Ph.D., NIMH
Alfred Hellman, Ph.D., NCI
G. Alderson, D.D.S., M.D., C. Boyers, D.D.S., L. Zimmerman, M.D., K. Ishak, M.D., F. Mostofi, M.D., L. Hockholder, M.D., and L. Johnson, M.D., AFIP
J. Sommers, M.D., I. F. Brody, M.D., D. Bigner, M.D., and J. Wagner, D.V.M., Duke University Medical Center
U. J. Lewis, Ph.D., Scripps Institute

COOPERATING UNITS: Environmental Biology and Chemistry Branch, NIEHS
Laboratory of Pathology, Viral Leukemia and Lymphoma Branch, Experimental Pathology Branch, and Viral Oncology Branch, NCI
Medical Arts and Photography Branch, NIH
Laboratory of Cerebral Metabolism, NIMH
Oral, Ophthalmologic, Orthopedic, Hepatic, Renal and Pulmonary Branches, AFIP
Animal Resources, Neurology and Pathology Departments, Duke University Medical Center
Scripps Institute, LaJolla, California

MAN YEARS

Total:	7.0
Professional:	3.0
Other:	4.0

OBJECTIVES: (1) To develop techniques for efficient breeding of the opossum under laboratory conditions, (2) to characterize the neonatal and developing opossum anatomically and physiologically and to determine their pathophysiologic responses to selected environmental toxins and carcinogens.

METHODS EMPLOYED: Special pharmacological techniques, light and electron microscopy, and standard biochemical methods of ultracentrifugation, thin-layer chromatography and gas chromatography.

MAJOR FINDINGS:

(1) Opossum Breeding. Manuscripts summarizing the data accumulated in this project are being prepared for publication.

(2) Opossum Film. Completion of the films entitled "The American Opossum as a Biomedical Model. I. The Neonatal and Developing Opossum" and " II. The Adult Opossum" has been delayed due to loss of technical support. An attempt will be made to obtain the remaining needed footage in January and February 1975, if technical support and pregnant animals are available.

(3) Biologic Characterization of the Opossum. (a) Studies to isolate and characterize growth hormone and prolactin from the opossum pituitary with Dr. U. J. Lewis, Scripps Institute, LaJolla, California, are being continued. The collaborator is in the process of analyzing pituitaries from 55 opossums supplied last year. There are no results to report to date. (b) A study of morphology and myogenic potential of the skeletal muscle of the opossum with Dr. Brody, Department of Neurology, Duke University Medical Center is being continued. No new results to report. (c) Light and electron microscopic studies of developing opossum skeletal and cardiac muscle with Dr. J. Sommers, Department of Pathology, Duke University Medical Center, are in progress. The collection of a developmental series which was delayed by unavailability of newborn opossums last year is now in progress. (d) Isolation and electrophoretic characterization of brain protein in the opossum with Dr. R. E. Martensen, Laboratory of Cerebral Metabolism, NIMH, are being continued. Brain samples from some 100 animals were supplied last year. There are no results to report.

(4) Carcinogenesis Study. The autopsy phase has been partially completed in the experiment in which ethyl nitrosourea was administered orally at 100 mg/kg as a single dose to opossums at ten stages of development. Gross photography and tissue processing for histologic study continues. Eye tumors developed in two experimental situations involving oral exposure of opossums to 100 mg/kg ENU at several different ages from birth to 16 weeks. A manuscript is in preparation. In the first experiment in 1971, in which ENU was given as a single dose, the eye tumor incidence was one out of 113. One female opossum exposed at 3 weeks of age developed a unilateral, intraoccular neoplasm. In the second experiment initiated in 1972 the same total amount of ENU was administered in four increments on an alternate day schedule. Under these conditions 4 of 42 animals developed tumors. One of the affected animals was exposed to ENU at one week of age; the three remaining animals were treated at two weeks of age.

Grossly the intraoccular neoplasms replaced the vitreous and involved the angle of the eye and retina. In late stages of growth, there was perforation of the sclera associated with inflammation and hemorrhage within the tumor and eventual rupture of the eyeball and sloughing of the intraoccular content. In one affected eye, however, there was massive extraoccular extention of the tumor without rupture of the globe. No extraoccular metastases were seen. There was, however, invasion of the lens capsule in all three eyes which remained intact. Histologically the tumors were classified as teratoid medulloepitheliomas (diktyomas). Heteroplastic tissue within the tumors compressed hyaline cartillage, rhabdomyoblasts, adipose tissue, and osteoid. The two latter tissues have not been reported in the human tumor. The intraoccular tumors are of interest because they represent the first reproducible intraoccular neoplasms to be induced by systemic exposure to a carcinogen, and may provide a useful model for the study of cancer of the eye.

Several additional unique tumors have been found in ENU treated animals during the past year including a ganglioglioma of the brain (tumors of the neuron have not previously been inducible in laboratory animals) and an islet cell adenoma of the pancreas.

Several new teratologic lesions have also been observed in opossums treated with ENU at birth. They include two cases of apparent hermaphrodism (characterized by completely developed penis, vaginae and uteri in association with ovotestes) and a small number of cases of bilateral or unilateral agenesis of the female internal genitalia.

The autopsy phase of the pilot study to compare the effects of intraperitoneal and intraoccular administration of ENU with those resulting from oral administration has been partially completed.

Pilot studies of three murine RNA viruses with Dr. M. Chirigos, Virus Leukemia and Lymphoma Branch, NCI, and a murine oncogenic DNA virus with Dr. C. Dawe, Laboratory of Pathology, NCI, in the newborn opossum have been negative with one exception. One animal exposed to polyoma virus subcutaneously at birth has developed a massive fibrosarcoma at the site of injection. Whether this is a spontaneous or induced lesion is unclear as yet. Virus titer determinations on surviving opossums in this study have been within the control animal range.

It has been necessary to abort the chromosomal studies of cancer bearing opossums due to loss of technical support. Tissue culture studies of selected tumors continue in collaboration with Dr. Wilna Woods, Virus Leukemia and Lymphoma Branch, NCI, at the NIH in Bethesda. There are no new findings to report from this work. The retrospective study to determine the state of organogenesis at the time of ENU induction of organ specific tumors has been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marsupial neonate is, in many respects, a fetus that completes the final portion of its development in an extra-uterine environment. Only in the marsupial is it possible to expose embryonic tissue *in vivo* to toxic insult without maternal metabolic or placental intervention and without effect of

the mother's own metabolism or the trauma of caesarian section. A systematic survey of the response of the marsupial to drugs and toxins with known pharmacologic or carcinogenic activity is not available. Such a study could indicate the value of the newborn marsupial for neonatal toxicology and experimental carcinogenesis and may yield new insight into the response of embryonic tissue to toxic insult. Preliminary findings indicate that the animal may have unique value as a model for studying mechanisms of carcinogenesis and indocrine development.

PROPOSED COURSE:

(1) Carcinogenesis Study. Histologic preparation of tissues and histopathologic interpretation and classification of lesions will continue. The current level of technical support and the anticipated loss of adequate laboratory resources will greatly delay completion of this work for publication.

(2) Opossum Film. Rough editing of footage available will be pursued as time permits in cooperation with MAPB, NIH. Completion of the film will depend on the availability of pregnant opossums and technical support.

Serial No.: NIEHS-ET-023
1. Environmental Toxicology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Chemical and Morphologic Studies of the Role of Lipid Metabolism in CNS Irritability"

PREVIOUS SERIAL NUMBER: NIEHS-PP-003

PRINCIPAL INVESTIGATOR: W. Jurgelsky, Jr., Ph.D., M.D.

OTHER INVESTIGATORS: P. M. Hudson, C. Boyd, F. Snyder, M.D., NIMN, and M. O'Connor, NINDS

COOPERATING UNITS: Section on Electroencephalography, NINDS
Laboratory of Psychobiology, NIMH

MAN YEARS

Total:	0.3
Professional:	0.1
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Disorders of steroid metabolism have been implicated among the causes of epilepsy. Previous studies, performed elsewhere by members of this laboratory, have established that an androgen-related, cholesterol-biosynthesis inhibitor, when administered once weekly to young marsupials (Didelphis marsupialis virginiana Kerr) in doses well below the toxic levels, induces a chronic major motor seizure state. These seizures can be induced at will by tactile stimulation. The opossum is the only laboratory species thus far tested that develops CNS hyperirritability in response to this drug. A number of organic phosphates and chlorinated hydrocarbon pesticides, which are known to influence steroid metabolism, also produce seizures at high levels of exposure. Alterations in lipid metabolism produced by this drug and other agents, such as pesticides with seizure-producing potential, will be correlated with changes in CNS irritability.

METHODS EMPLOYED: Optical microscopy, electron microscopy, thin-layer chromatography, electroencephalography, ultra centrifugation, gas chromatography and scintillation spectroscopy were used. Comparative studies were performed in rats and opossums.

MAJOR FINDINGS: As part of a comparative study on the effect of cholesterol biosynthesis inhibitors on CNS irritability, opossums (Didelphis virginiana

Kerr) were given 100 mg/kg Triparanol subcutaneously once weekly for six to nine months beginning at four months of age. Drug administration was associated with a significant growth retardation as compared to controls as well as bone deformities compatible with rickets. Serum calcium, phosphorous and cholesterol levels were reduced by approximately 30, 50, and 70%, respectively, from the control range and serum alkaline phosphatase levels were markedly elevated. There were no morphological alterations in the small intestine to support malabsorption as a mechanism for the rickets. The livers of Triparanol-treated animals, however, showed moderate to marked fatty degeneration of hepatocytes suggesting alterations in hepatic hydroxylation of Vitamin D as a possible cause of the rachitic changes. The results imply that the opossum may be a good animal model for studies of the intricacies of Vitamin D metabolism in the mammal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The induction of seizures in the marsupial by administration of an inhibitor of cholesterol biosynthesis represents a previously-unreported easily reproducible model for experimental chronic epilepsy. The apparent direct implication of alterations in steroid biosynthesis in the seizure state in these animals may offer a new window on neural irritability in general and on epilepsy in particular.

PROPOSED COURSE: Experiment terminated. Available data on the experimental epilepsy continues to be summarized for publication. A paper entitled "Rickets Associated with Triparanol Administration in the Opossum" has been submitted for publication.

PUBLICATION

Jurgelski, W., Jr., Hudson, P. M., and Vogel, F. S.: Induction of a chronic somatosensory epilepsy in the opossum (Didelphis virginiana Kerr) with an inhibitor of cholesterol biosynthesis. Brain Research. 64: 466-471, 1973.

PHARMACOLOGY BRANCH

PHARMACOLOGY BRANCH
Summary Statement

The Pharmacology Branch has four general research programs: (1) toxication-detoxication systems and their relation to organ damage by chemicals, (2) pharmacokinetics and the use of such approaches for predicting and modifying chemical uptake, storage and release by body tissues, (3) lung physiology and biochemistry and the use of such studies to detect early damage to the lung or understand the mechanisms of lung response to chemicals, and (4) inhalation exposures and toxicology and the study of how to mimic realistic exposures of animals to pollutants and mixtures of pollutants and understand those interactions which may produce synergism or antagonism of effects of inhaled materials. These research programs have several goals among which are an explanation of why chemicals may damage one organ more than another, one species or age of animal more than another, and the devising of better methods of detecting early toxicity of chemicals before irreversible changes occur.

Among the many ways to apply pharmacologic principles and technics to studies of possible adverse effects of environmental components, we have chosen those we feel best utilize the strengths of our staff and offer the best chance for mutual interactions and interdisciplinary approaches with scientists both within our Branch and in other Branches here at NIEHS and elsewhere (e.g., at N.C. State University, UNC and Duke University). Thus our marine pharmacology program uses input from the toxication-detoxication group as well as the pharmacokinetic group in studying why some fish accumulate pollutants and others do not and why some organs accumulate pollutants and others do not. This program has also interacted with scientists from other Branches here at NIEHS and other institutes of NIH (NCI), as well as many universities. In studies of lung uptake, storage and release of chemicals, all four program areas of our Branch are involved together with scientists from NIH (NCI, NHLI) and many universities (Cornell, Maryland, Iowa, Yale, Cincinnati, Duke).

Program

Toxication-Detoxication Systems: Chemical metabolisms by lung, gut, liver and skin are being characterized with respect to substrates, inhibitors, methods for *in vitro* assay of enzyme activities, response to inducers, etc. The balance between toxication (e.g. - epoxidation) and detoxication (e.g. - glutathione conjugation) reactions in various tissues is being studied in some detail. Factors influencing this balance between toxication and detoxication may include heavy metals, age, or enzyme inducers and the effects of such imbalance on toxicity of chemicals applied by various routes is being assessed.

These studies seek to define mechanisms and time course of pollutant transfers in marine tissues as one step in understanding sources of pollutant concentrations and exposures for man.

Pharmacokinetics: This program seeks to define the uptake, storage and release of chemicals by animal tissues; to study interactions of chemicals and drugs at sites of absorption, distribution and storage of chemicals; and to construct models of chemical disposition so as to predict the time course of a new chemical's stay in the body. Studies have begun with single PCB isomers and with one mammalian (rat) and two marine species (lobster, dogfish shark). Collaboration with Drs. Dedrick, Zaharko and Bischoff (Cornell, Maryland and NIH) in planning this research and in constructing these models, as well as the help of several people from our own Biometry Branch (especially Dr. Anderson), have been vital to the progress made. The isolated perfused lung is being studied as part of this program to better understand specific tissue localizations of chemicals and the mechanisms for interactions of chemicals at tissue stores. Competition for both uptake and release of chemicals by the lung has been demonstrated and may represent a significant toxicologic problem.

Lung Physiology, Biochemistry and Pharmacology: Studies on the chemical and biochemical composition of the lung concentric lamellar body and comparisons with the composition of lung surfactant and lung washout fractions suggest a precursor-product relationship. Changes in protein composition of lung washout fractions in lung proteinosis diseases are being studied in collaboration with scientists at Duke University. Incorporation of chemicals into lung coenzymes and incorporation of chemicals and drugs into peptides and tissue proteins may represent mechanisms for tissue fixation and storage of chemicals as well as serving as a source of allergens to trigger respiratory and systemic diseases. These processes of chemical incorporation are being studied in some detail in our Branch. Lung lipid metabolism (surfactant synthesis, prostaglandin metabolism) and lung macrophage composition and function are also being investigated by several groups. Effects of pollutants (especially oxidants) on these processes have been studied in a few cases and will be investigated in more detail.

Inhalation Toxicology: The inhalation toxicity of common solvents and the effects of animal pretreatment with modifiers of the metabolism of these solvents continues to be a major program. Benzene and xylene inhalation toxicity are modified in different ways by animal treatment with inducers of hepatic metabolism of these chemicals. The ability of dichloromethane to cause blood CO-hemoglobin levels to increase is also affected by some but not all manipulations of the hepatic microsomal drug-metabolizing systems. Species differences in solvent toxicity and the metabolism of solvents by extrahepatic tissues is being studied in an attempt to correlate these two parameters. The toxicity of inhaled freons for the heart conduction systems has been studied in both normal and cardiomyopathic hamsters. The use of this model disease state to show that common chemicals can be toxic in "sick" but not "well" animals is an important approach we hope to continue. The lung lesion produced by acute and chronic exposure of animals to spray can anti-perspirants is being studied for its dose-response and reversibility characteristics.

Sciences. Drs. Bend and Hook have both been reviewers for Biochemical Pharmacology and Chemico-Biological Interactions. Dr. Bend has recently been elected to membership in the American Society for Pharmacology and Experimental Therapeutics.

Dr. Curtis Harper is an Adjunct Assistant Professor of Pharmacology at the School of Medicine, University of North Carolina at Chapel Hill and lectures to medical and graduate students in departmental courses in pharmacology. Dr. Harper recently presented a seminar at the University of North Carolina at Chapel Hill (March, 1974).

Dr. Matthews presented a paper by invitation at the American Chemical Society Meeting, Los Angeles, California, April, 1974.

Serial No.: NIEHS-P-001
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of these Systems"

PREVIOUS SERIAL NUMBER: NIEHS-PT-032

PRINCIPAL INVESTIGATORS: T. Devereux, M.S., J. R. Fouts, Ph.D.

OTHER INVESTIGATORS: R. M. Philpot, Ph.D.

COOPERATING UNITS: Environmental Biometry Branch, Chemistry

MAN YEARS:

Total:	2.0
Professional:	1.5
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: This is a general effort directed to classifying and understanding the actions of chemicals and various physiological factors that affect the body mechanisms for metabolizing "xenobiotics" and other substances (endogenous and exogenous) which can be substrates for systems often called "drug-metabolizing enzymes." Such metabolizing enzyme systems are found relatively concentrated in excretory organs and tissues found at the interface between man and his environment (e.g., skin, gut, lung, kidney, and liver). These enzymes respond to a variety of chemicals (such as pollutants, pesticides, food additives) and changes in physiology (age, sex, nutrition) by increases or decreases in their activity which can affect the action and toxicity of xenobiotics that reach these enzymes. Combinations of chemicals can affect these xenobiotic metabolizing systems in ways both qualitatively and quantitatively different from effects of single entities. Present efforts are devoted to studies on the rates of development of these systems in the rabbit fetus and newborn - especially comparing toxifying and detoxifying systems in liver and lung as well as the effects of metal ions on these systems in the young animal. Levels of these enzymes in the liver and lung of pregnant rabbits are also being studied. Mechanisms of metal ion effects on the N-oxidase system in lung are also under investigation.

METHODS EMPLOYED: Most work has been done on liver and lung microsomal preparations though this was preceded in the case of lung with studies on other parts of the homogenate. Microsomes are isolated from tissues of animals of different ages (rabbits) and used to study metabolism of different xenobiotics and drugs or the amount or activity of key steps in the MFO-P450 dependent system. Selected toxifying systems have been partially purified from lung and liver by classical technics of enzyme purification - sonication, solubilization with detergents, concentration by selective precipitation with $(NH_4)_2\ SO_4$, etc. Spectrophotometric methods are used to study cytochromes (B_5, P-450 and their oxidation and reduction), and quantify metabolites of substrates added to microsomes or purified enzyme systems.

MAJOR FINDINGS: Age-dependent changes in toxifying-detoxifying systems in liver and lung: This extends a study now in its third year and has concentrated on the metabolism of a model substrate by two mechanisms - N-demethylation and N-oxidation. In rabbits, the demethylase was found in trace amounts in the liver and lung of 28-day (gestation age) fetuses. Demethylase activities increased somewhat at birth and increased rapidly to adult levels between two and four weeks after birth. N-oxidase activity was present in both tissues at 24 days fetal gestation age and increased steadily to adult levels at 4-6 weeks postpartum. In the newborn rabbit lung, microsomal N-oxidation is stimulated by Hg^{++}, although not to the extent seen in the adult. However, there was no stimulation by 0.1 mM Hg^{++} seen with the N-oxidase activity from lung microsomes of the 28-day fetus.

Increased levels of N-demethylase and N-oxidase activities were seen in the lung microsomes of rabbits pregnant for 28 days. However, no difference was seen in these enzyme levels when liver microsomes from 28-day pregnant and nonpregnant rabbits were compared. There was also no sex difference in liver or lung N-demethylase or N-oxidase activities between adult male and nonpregnant female rabbits. However, during the latter third of pregnancy, cytochrome P-450 concentration as well as demethylase and N-oxidase activities increased in lung microsomes of pregnant rabbits to as much as double control (unpregnant) levels.

Effect of metal ions on N-oxidation in lung and liver microsomes: This is a continuing study on the mechanism of metal ion stimulation of N-oxidation in the rabbit lung. The solubilized and partially purified rabbit lung N-oxidase was studied to learn why the stimulation by Hg^{+2} of the lung N-oxidase disappeared in the solubilized preparation, whereas the stimulation by Mg^{+2} remained. A heat stable, dialyzable factor seems to be involved and attempts are being made to identify this factor.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This research project seeks to understand some of the factors that affect xenobiotic metabolism in tissues in contact with the environment. Comparisons of lung and liver systems have been made to see where differences exist and what these might mean in the toxication-detoxication mechanisms of the body. The sex and age-related changes in toxication versus detoxication

pathways that are being observed may aid our identifying and understanding stages in life when the body is more susceptible to toxic agents. These studies, as well as those looking into the mechanisms of action of metal ions on N-oxidation in lung and livers and the comparisons of similar enzyme systems (e.g., N-hydroxylation vs. N-oxidation) in different tissues, lead us to a better understanding of the balance between toxication and detoxication mechanisms and the varied ways chemicals can alter these systems and this balance.

PROPOSED COURSE: Further study on the effects of metal ions on fetal microsomal enzymes. Attempt to induce lung microsomal enzymes in adult male and female rabbits with substances (e.g., testosterone, corticosteroid) known to be at high levels in the pregnant rabbit during the latter part of pregnancy. Further purification and study of liver and lung N-oxidases to identify components in lung which account for metal ion stimulation of this system. Study of N-hydroxylation of primary and secondary amines to compare mechanisms of this oxidation with N-oxidation of tertiary amines by lung vs. liver.

PUBLICATIONS

Devereux, T. R. and Fouts, J. R.: N-oxidation and demethylation of N,N-dimethylaniline by rabbit liver and lung microsomes. Effects of age and metals. Chemico-Biological Interactions 8: 91-105 (1974).

Fouts, J. R., Bend, J. R., James, M. O., and Devereux, T. R.: Perinatal development of N-oxidase, N-demethylase, epoxide hydrase and glutathione (GSH) S-epoxide transferase in hepatic and extrahepatic tissues of guinea pigs and rabbits. In Procs. International Symposium on Perinatal Pharmacology, in press, 1974.

Serial No.: NIEHS-P-002
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Arylhydroxylases of Lung"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: C. Harper, Ph.D., R. T. Drew, Ph.D., and G. E. R. Hook, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., L. B. Gilmore, B.A., F. Harrington, E. Lores, B.S., M. T. Riley, and J. L. Schroeder

COOPERATING UNITS: Environmental Biology and Chemistry Branch (Histology and Electron Microscopy)

MAN YEARS:

Total:	0.3
Professional:	0.2
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: To investigate in detail the arylhydroxylases of lung using a number of simple aromatic compounds as substrates. Because some of these compounds (e.g., halogenated benzenes) are known to cause liver necrosis, their effects on the lung after inhalation will be investigated morphologically as well as enzymically.

METHODS EMPLOYED: Microsomes were isolated from lung. Arylhydroxylase activities are assayed using radiochemical methods. Morphological investigations involve both light and electron microscopic methods.

MAJOR FINDINGS: Rabbit lung microsomal preparations were more active in metabolizing benzene, bromobenzene and chlorobenzene than the liver preparation. Conversely liver microsomes metabolize aniline at a faster rate than lung microsomes. Nitrobenzene does not appear to be hydroxylated under the conditions used in these metabolic studies. The rates of metabolism of the four substrates (benzene, aniline, bromobenzene and chlorobenzene) are predicted by the electronegativity of the substituent group; i.e., the plot of log Vmax vs. electronegativity gives a straight line with both liver and lung microsomes from rabbits.

Rabbits exposed to bromobenzene (1,000 ppm, three hours per day for three days) were not found to have necrotic lungs or liver when sacrificed 24 hours after the final exposure. The exposure did not alter the rate of *in vitro* metabolism of bromobenzene and chlorobenzene by lung and liver microsomal preparations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most of the substrates are either materials of environmental concern (e.g., benzpyrene and biphenyl) or are used commercially. Some of them are known to cause liver necrosis but their effects on lung are not extensively studied as yet.

PROPOSED COURSE: These investigations will be coupled with an investigation of the sulfokinases responsible for the sulphation of the products of arylhydroxylation.

Serial No.: NIEHS-P-003
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Hepatic and Extrahepatic Conjugating Pathways and the Effects of Environmental Contaminants and Other Xenobiotics on the Enzymes Involved"

PREVIOUS SERIAL NUMBER: NIEHS-PT-015

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., M. O. James, Ph.D., and F. C. P. Law, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., G. W. Lucier, Ph.D. (Env. Toxicology Branch), G. L. Foureman, B.S., L. B. Gilmore, B.S.

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS:

Total:	2.5
Professional:	1.7
Other:	0.8

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are (1) the characterization of some microsomal, mitochondrial and soluble conjugating and hydrating enzymes of extrahepatic tissues as compared to the liver enzymes, (2) to determine the effects of selected xenobiotics upon these enzymes, (3) to assess the relative importance *in vivo* of this extrahepatic enzyme activity as compared to the liver, following ingestion or administration of chemicals and drugs (substrates) via various routes including inhalation, (4) to investigate the perinatal development of some conjugating and hydrating enzymes in a few species, and (5) to study the combined effects of several compounds (which have as a common step in their metabolism a glutathione-dependent reaction) on *in vivo* toxicity and on extrahepatic tissue necrosis by these chemicals.

METHODS EMPLOYED: Differential centrifugation, spectroscopic, fluorometric and radiochemical analyses.

MAJOR FINDINGS:

1. A radiochemical procedure, which measures product formation, was developed for assay of soluble fraction glutathione (GSH) S-epoxidetransferase activity toward ^{14}C-8-styrene oxide. Of several tissues studied in the

rabbit, liver soluble fraction was found to have the highest GSH S-epoxide-transferase specific activity, followed in turn by kidney (1/3 liver activity), lung (1/4 liver activity) and small intestinal mucosa (1/8 liver activity). Microsomal epoxide hydrase activity in the rabbit was found to be highest in the liver with microsomes from small intestinal mucosa possessing up to 50% of liver activity. Kidney and lung possessed low, but detectable, epoxide hydrase activity toward styrene oxide. In terms of total activity, liver contained much more GSH S-epoxidetransferase and epoxide hydrase than did any other tissue studied.

Another radiochemical assay was developed to measure N-acyltransferase activity. In this procedure the formation of phenylacetylglycine from [^{14}C-carboxy]-phenylacetyl coenzyme A (in the presence of enzyme and excess glycine) is monitored. In the rabbit, kidney mitochondria were found to contain the highest conjugating activity although liver mitochondria and liver and kidney soluble fractions also catalyze this reaction. The general enzymological properties of the mitochondrial enzyme, which is evenly distributed between kidney cortex and medulla, are under investigation.

2. The pretreatment of rabbits with 3-methylcholanthrene (3-MC), phenobarbitone (PB) or pregnenolone-16α-carbonitrile (PCN) did not lead to increased levels of microsomal epoxide hydrase activity in liver or lung. However, rabbit hepatic cytochrome P-450 levels were elevated after both 3-MC or PB administration. There was also no increase in rabbit liver or lung GSH S-epoxidetransferase after PB or PCN pretreatment.

3. The developmental patterns of microsomal epoxide hydrase and soluble fraction GSH S-epoxidetransferase were studied in the guinea pig. At the earliest time point studied (38 days of gestation) all tissues had significant transferase activity. The specific activities at this time were about 80% (lung), 45% (gut), 20% (liver) and 15% (kidney) of those found in adults. Specific transferase activity of placenta decreased with increasing gestational age, whereas placental epoxide hydrase activity was low regardless of age. In liver, epoxide hydrase activity was first detected at 44 days of gestation (10% adult activity); it developed rapidly in the first week after birth and reached adult levels near one month of age.

4. The effect of administering diethyl maleate (DEM) to rats on hepatic and extrahepatic glutathione levels has been determined using a very sensitive fluorimetric procedure, adapted from the published method of Cohn and Lyle (Anal. Biochem. 14: 434, 1966). DEM (IP or oral) at 600 mg/kg significantly depletes lung and liver glutathione levels, but does not affect kidney GSH. At similar doses, lung GSH is depleted substantially more by IP treatment than by oral dosing. Maximum depletion (87%) of liver glutathione occurs about 30 minutes after IP administration of DEM (600 mg/kg).

Certain organophosphate insecticides, such as Dichlorvos and Guthion, are known to be metabolized in mammals, at least partially, by GSH-dependent pathways. Therefore, the effect of parenteral DEM treatment (to liver hepatic and pulmonary GSH levels) on the toxicity (LD_{50}) of Dichlorvos or Guthion was measured. In both cases GSH depletion increased the toxicity (i.e. lower LD_{50}) of the organophosphate compound slightly but not dramatically.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The conjugation of xenobiotics or their metabolites which are chemically reactive (e.g., epoxides and N-oxides) by liver, lung, gut and other extrahepatic tissues may play a significant role in the overall toxicity of agents which enter the body via the pulmonary system or the alimentary canal. The developmental patterns of conjugation pathways active in detoxication are of importance since these may determine the susceptibility of the fetus and neonate to environmental contaminants, and may also be related to chemical teratogenesis. In the real-life situation one is very rarely exposed to a single xenobiotic; rather one comes in contact with a large number of synthetic organic chemicals including food additives, drugs, plasticizers, insecticides, etc. For this reason investigations concerning interactions between more than one foreign compound are of relevance to environmental health.

PROPOSED COURSE:

1. The relative importance of epoxide hydrase and GSH S-epoxidetransferase, two enzymes which are active in the metabolism of epoxides, in the biotransformation of epoxides will be assessed by determining the relative amounts of glycol and GSH conjugate formed by 10,000 x g supernatant fractions of various tissues.

2. The developmental pattern of epoxide hydrase, GSH S-epoxidetransferase, GSH S-aryltransferase, phenylacetyl N-acyltransferase and glucose-6-phosphate dehydrogenase will be followed in liver, lung, kidney, intestine and placenta of the rabbit.

3. The effect of diethyl maleate administration to rats on the toxicity of other organophosphate insecticides will be assessed.

4. The metabolism of ^{14}C-styrene oxide will be examined in the isolated perfused rabbit lung preparation.

PUBLICATIONS

James, M. O., Bend, J. R., and Fouts, J. R.: Hepatic and extrahepatic metabolism of epoxides in the rabbit. The Pharmacologist 15: 191, 1973.

Bend, J. R., Hook, G. E. R., and Fouts, J. R.: Hepatic and extrahepatic glutathione S-aryltransferase activity in the rabbit. The Pharmacologist 15: 249, 1973.

Bend, J. R., James, M. O., and Fouts, J. R.: Perinatal development of glutathione S-epoxidetransferase (ETase) and epoxide hydrase (EH) in liver and selected extrahepatic tissues of the guinea pig. Fed. Procs., Vol. 33 (3), p. 566, 1974.

Serial No.: NIEHS-P-004
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Species Differences in Stimulation of Toxication-Detoxication Systems by Environmental Components. A Comparative Study of the Biochemical Properties of Xenobiotic-Metabolizing Enzymes in Liver and Intestine of Rabbit"

PREVIOUS SERIAL NUMBER: NIEHS-PT-031

PRINCIPAL INVESTIGATOR: R. S. Chhabra, Ph.D.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., R. J. Pohl, B.S., and J. Van Anda, B.S.

COOPERATING UNITS: Environmental Biometry Branch, Environmental Biology and Chemistry Branch

MAN YEARS:

Total:	1.6
Professional:	1.0
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: To study the effects of various physiological (age, sex, nutrition, hormones), genetic (animal species, strain) and environmental (exposure to insecticides, carcinogenic polycyclic hydrocarbons) factors on the enzymes metabolizing xenobiotics in various tissues.

There are three projects currently under study: (1) Hormonal regulation of induction of hepatic xenobiotic-metabolizing enzymes by insecticides and polycyclic hydrocarbons in rats and mice; (2) sex differences in xenobiotic metabolism by extrahepatic tissues in rats (lung, gut and kidneys); and (3) comparative study of xenobiotic-metabolizing enzymes in liver and intestine of rabbits.

METHODS EMPLOYED: Microsomal fractions were isolated by differential centrifugation. Spectrophotometric and spectrophotofluorometric techniques were used for the assays of xenobiotic-metabolizing enzymes.

MAJOR FINDINGS:

1. Differences in hormonal regulation of hepatic xenobiotic-metabolizing enzymes in rats and mice: In our previous study we found that the relative resistance shown by mice to hepatic enzyme induction could be overcome by high dose levels of DDT or benzpyrene (BP) (100 mg/kg and higher) and especially by treatments of longer duration than used before. The inducing effect of DDT or BP is short; seen at 24 hr but not at 96 hr after last injection. In rats, the maximal effect of both enzyme inducers was relatively long-lived after termination of the treatment with inducers. In contrast to rats, BP treatment in mice did not shift the hepatic microsomal reduced cytochrome P-450-CO absorption spectral peak from 450 nm to 448 nm. The pH optima for hepatic microsomal aniline hydroxylase was also not shifted by BP treatment of mice in contrast to the pH optimum shift seen after BP treatment in rats. Studies on hormonal regulation of ethylmorphine-N-demethylase, aniline hydroxylase and BP hydroxylase in mice indicated that adrenalectomy, castration or adrenalectomy plus castration do not lower the basal (uninduced) activities of these enzymes (in rats with similar operations, basal drug-metabolizing enzyme activities are lowered by 50-90 percent depending on the enzyme). Also the magnitude of stimulation of hepatic xenobiotic-metabolizing enzymes by pretreatment of mice with DDT, BP, 3-MC or phenobarbital in mice without adrenals, testes or both was not less than in sham-operated control mice (as does occur in rats) but instead was somewhat higher. These findings suggest that there is a species variation in hormonal regulation of hepatic xenobiotic metabolism in rats and mice.

2. Sex differences in xenobiotic metabolism by extrahepatic tissues in rats: Hepatic microsomes from male rats metabolize xenobiotics faster than hepatic microsomes from female rats. Sex differences in the metabolism of xenobiotics may be specific to only some organs or tissues since enzyme preparations from rat lung and gut did not show sex differences in the metabolism of various drug substrates. Rat kidney microsomes exhibited sex differences in rates of hydroxylation of BP and glucuronidation of p-nitrophenol though, in contrast to liver, the rates of metabolism were higher in kidneys from female than from male rats. The sex differences in both hepatic and renal microsomal UDP-glucuronyl transferase activity existed even after maximal activation of the enzyme by Triton X-100.

3. Comparative study of xenobiotic-metabolizing enzymes in liver and intestine of rabbit: (A part of this study was in collaboration with Ms. Roberta J. Pohl.) We used rats, mice, hamsters, guinea pigs and rabbits in a preliminary study to select the model animal for more detailed study of xenobiotic-metabolizing enzymes in gut. The xenobiotic-metabolizing enzymes studied were present in livers of all the species but activities varied over a wide range between species. In intestines from mice, rats, guinea pigs and hamsters, either some of the enzymes were absent or had very low activity which would require very sensitive methods for detection. Rabbit emerged as the best animal model to study xenobiotic metabolisms in gut because all xenobiotic-metabolizing enzymes studied were present in easily measurable quantities.

A detailed study of xenobiotic-metabolizing enzymes in liver and gut of rabbit is presently under investigation. A distribution study of some of the xenobiotic-metabolizing enzymes along the entire length of small intestine showed that maximum activity of these enzymes is located in the proximal 24 inches of the gut. Rabbit gut microsomal aniline hydroxylase, BP hydroxylase and aminopyrine-N-demethylase required reduced nicotinamide adenine dinucleotide phosphate and oxygen for maximum activity. In general the protein vs. activity, time vs. activity, substrate concentration vs. activity and pH vs. activity curves of aniline hydroxylase, BP hydroxylase and aminopyrine-N-demethylase were similar in microsomes from gut and liver. Cytochrome c, SKF 525-A and CO inhibited microsomal mixed-function oxidase in gut as well as in liver.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Species differences in metabolism of and response to pollutants by xenobiotic-metabolizing systems may explain both species specific toxicities of pollutants and interactions of pollutants with drugs and chemicals. Pollutants in food and water as well as air-contact gut and gut enzymes may be a first line of defense of the body against such materials.

PROPOSED COURSE: Studies on response to stimulation or inhibition of xenobiotic metabolism and on the hormonal regulation of this metabolism in the livers of mice vs. rats has been completed. A study of response of various tissue's xenobiotic metabolisms to inducers such as BP, 3-MC, dioxins, DDT and phenobarbital in hepatic enzyme-induction resistant (DBA) vs. nonresistant mice (C57BL) has begun.

The characterization of gut xenobiotic-metabolizing enzymes by study of optimal conditions for in vitro assays - e.g., temperature, pH, cofactor and substrate concentration will soon be completed. This will be followed by study of the effect on gut xenobiotic metabolisms of treating rabbits with various enzyme stimulators (such as DDT, BP, 3-MC, dioxins) and inhibitors (e.g., Hg). Changes in enzymes with age, deficient diet, time of day (diurnal variations) and stress on different xenobiotic-metabolizing enzymes in the rabbit gut will be studied.

PUBLICATIONS

Chhabra, R. S., and Fouts, J. R.: Stimulation of hepatic microsomal drug-metabolizing enzymes in mice by 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) and 3,4-benzpyrene. Tox. Appl. Pharmacol. 25: 60-70, 1973.

Chhabra, R. S. and Fouts, J. R.: Stimulation of hepatic drug-metabolizing enzymes by DDT, polycyclic hydrocarbons or phenobarbital in adrenalectomized and/or castrated mice. Tox. Appl. Pharmacol. 28, 1974 (in press).

Chhabra, R. S. and Fouts, J. R.: Further studies on induction of hepatic microsomal drug-metabolizing enzymes (HMDME) in normal, adrenalectomized and/or castrated mice. Pharmacologist 15: 111, 1973.

Serial No.: NIEHS-P-005
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Microsomal Drug and Chemical Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: R. S. Pohl, B.A., J. R. Fouts, Ph.D., R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: None

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS:

Total:	0.8
Professional:	0.6
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: To isolate microsomal fractions from skin, characterize the xenobiotic-metabolizing enzymes associated with those fractions and elucidate the role of these enzymes in toxic responses of skin to environmental agents.

METHODS EMPLOYED: Of a variety of skin disruption and homogenizing techniques tried, the greatest microsomal yield and retention of cytochrome P-450 was accomplished with Polytron disruption of 0.5 g aliquots of minced skin by four, 5-second bursts at about 15,000 rpm followed by homogenization by five strokes of a conical Teflon pestle in a fitted glass vessel. Microsomal fractions were isolated by standard procedures. Cytochrome P-450 content was estimated from reduced-CO difference spectra. 7-Ethoxycoumarin de-ethylating activity was measured by recording the appearance of the fluorescent metabolite, umbelliferone. Benzpyrene hydroxylase activity was measured by the standard fluorescent method.

MAJOR FINDINGS: Difference spectroscopy of microsomal fractions prepared from the skin of male CD-1 mice showed evidence of some contamination with hemoglobin, methemoglobin and mitochondrial pigments as well as the presence of substantial amounts of P-420. However, an estimated 0.05 nmoles of

cytochrome P-450 was detectable per mg microsomal protein. Enzyme turnover in picomoles/min/nmole cytochrome P-450, based on these cytochrome values, were: 7-ethoxycoumarin de-ethylase, liver 2000, skin 1500: benzpyrene hydroxylase, liver 500, skin 250.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Skin comes in contact with a large number of foreign chemicals in cosmetics, cleaning compounds, air and water pollution, synthetic and easy-care fabrics, and occupational environments. Both localized and systemic toxicity of these chemicals may be mediated by the balance of toxicating and detoxicating mechanisms in the skin, including xenobiotic-metabolizing enzymes. The study of these enzymes and their responses to both chemical and physical factors will lead to a better understanding of the total body protective systems toward foreign chemicals.

PROPOSED COURSE: The extent to which cytochrome P-450 degradation to cytochrome P-420 might be an artifact of the disruption-homogenization process will be evaluated by assaying MFO activity in skin homogenates prepared by various means and by investigating procedures to further stabilize the cytochrome during the procedure. The *in vivo* activity of the microsomal enzyme system will then be characterized, after which a systematic study will be made of the effects of MFO inhibitors and inducers (many of which are environmental contaminants), age, sex and species, and physiological stresses on enzyme activities and their response to environmental chemicals.

Serial No.: NIEHS-P-006
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "In Vitro Xenobiotic Metabolism by Selected Marine Species and the Effects of Environmental Pollutants on these Biotransformations"

PREVIOUS SERIAL NUMBER: NIEHS-PT-034

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., M. O. James, Ph.D., R. J. Pohl, B.A.

OTHER INVESTIGATORS: J. R. Fouts, Ph.D., G. L. Foureman, B.S., T. R. Devereux, M.S.

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS:

Total:	0.8
Professional:	0.6
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: (1) To compare microsomal xenobiotic oxidative metabolism in several vertebrate and invertebrate marine species, (2) to characterize the hepatic mixed-function oxidase system of the little skate, Raja erinacea, since it possesses relatively high enzyme activity, (3) to investigate some xenobiotic conjugating enzymes in marine vertebrates and invertebrates, and (4) to study the effects of environmental contaminant administration on in vitro xenobiotic mixed-function oxidation and conjugation by tissues from marine elasmobranchs and teleosts.

METHODS EMPLOYED: Subcellular fractions are prepared from tissue homogenates of freshly collected marine specimens. Standard procedures are utilized for the determination of enzyme activities and quantitation of microsomal cytochromes.

MAJOR FINDINGS:

1. Mixed-function oxidase activity toward aniline, d-benzphetamine, 7-ethoxycoumarin and benzpyrene of microsomes from tissues of various crustacea, elasmobranchs, teleosts and a single cyclostome from Maine was

determined. No activity was detectable in rock crab or lobster hepatopancreas, possibly due to inhibition by digestive juice. Activity (per mg microsomal protein) in the other species was generally about one-tenth that of rat liver microsomes although cytochrome P-450 content was about 25 percent that of rat liver microsomes. Kidney was the only extrahepatic tissue of the little skate found to contain significant mixed-function oxidase activity. Renal microsomes from the dogfish shark (Squalus acanthias) also possessed considerable xenobiotic-metabolizing activity with the substrates studied.

2. Several characteristics of the skate liver microsomal xenobiotic-metabolizing system are very similar to those of the analogous mammalian systems. Reduced NADPH and molecular oxygen were required for maximum activity; cytochrome c, CO, SKF 525-A and metyrapone are all effective inhibitors of mixed-function oxidase activity; and cytochrome b_5 and cytochrome P-450 are constituents of skate liver microsomes.

The temperature optimum of 7-ethoxycoumarin O-deethylation by skate liver microsomes was shown to be 30° C. As incubation temperatures were increased above the optimum, product formation began to fall off. This was especially true at 41° C (the highest temperature studied), where metabolism had virtually ceased after 15 minutes. When incubations were conducted at the approximate summer temperature of Frenchman Bay, Maine (12° C), specific activity was only 37 percent that recorded at the in vitro temperature optimum.

3. The distribution of glutathione (GSH) S-aryltransferase activity toward 1,2-dichloro-4-nitrobenzene was determined in the soluble fraction of liver or hepatopancreas from several vertebrate and invertebrate marine species. All species monitored, including crab and lobster, contained significant enzyme activity. The killifish, a small teleost, had the highest specific activity observed (45 nmoles product formed/min/mg soluble protein). This activity is about fivefold that found in rabbit liver. GSH-S-aryltransferase was widely distributed in extrahepatic tissues of the little skate. In fact, soluble fractions prepared from spiral valve mucosa and stomach lining possessed higher specific activity and more total activity per gram mucosa than did liver. Pancreas, kidney, gill and spleen also possessed detectable activity, whereas heart did not.

4. Phenobarbital (PB), Aroclor 1254 or 3-methylcholanthrene (3-MC) was administered to skates and the activities of the hepatic microsomal mixed-function oxidase system were determined. All of these compounds are potent inducers of analogous systems of rat liver. However, IV, IP, or oral pretreatment of skates with PB resulted in significant inhibition of xenobiotic-metabolizing activity in skate hepatic microsomes, which is probably related to the presence of unchanged PB or its metabolite(s) in these microsomes at the time of assay. PB treatment did not increase the cytochrome P-450 content of skate hepatic liver microsomes (as compared to nontreated control animals).

Large oral doses of Aroclor 1254 (100 mg/kg for 3 consecutive days) given to skates at least 48 hours before sacrifice also did not induce benzphetamine demethylase or aniline hydroxylase activities of isolated hepatic microsomes. However, statistically significant increases in hepatic microsomal aniline,

benzphetamine and benzpyrene metabolisms were recorded in skates that were pretreated orally with 3-MC. This polycyclic hydrocarbon was also found to induce 7-ethoxycoumarin O-deethylase and benzpyrene hydroxylase activity of liver microsomes of the winter flounder when administered orally to flounder 3½ days before sacrifice.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminated with substantial amounts of pesticides, industrial by-products and other synthetic organic chemicals which are produced in huge quantities by our technologically-orientated society. The ability or inability of marine species, particularly those that are edible or those which are important in the aquatic food chain, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and to their potential value and danger as direct and indirect foodstuffs for man.

PROPOSED COURSE:

1. The time-course, dose-response and duration of the apparent 3-MC-caused enzyme induction and the substrate specificities of the induced enzyme systems of little skate liver microsomes will be studied after oral administration of 3-MC. Moreover, the qualitative nature (as determined from binding spectra - e.g., cytochrome P-450 or P-448) of the CO-binding hemoprotein and the amount present in hepatic microsomes will be compared in control vs. treated (induced) skates.

2. Microsomal epoxide hydrase and GSH-S-epoxidetransferase activities will be studied in liver and several extrahepatic tissues of the little skate. The GSH content of various skate tissues will also be determined and the effects of administration of known GSH depletors such as diethyl maleate and styrene oxide upon the GSH content of various skate tissues will be studied.

3. A preliminary study of the fate of phenylacetic acid (PAA) in the marine species indicated that an amino acid other than glycine is utilized for conjugating PAA prior to urinary excretion. This amino acid conjugate will be identified and the formation of this metabolite *in vivo* and *in vitro* will be investigated in more detail.

4. The response of the little skate microsomal mixed-function oxidase and glucuronyl transferase systems to pretreating skates with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most potent inducer of mammalian microsomal enzymes yet discovered, will be determined. Microsomes from both kidney and liver of treated skates will be investigated.

PUBLICATIONS

Bend, J. R., Pohl, R. J., and Fouts, J. R.: Some properties of the microsomal drug-metabolizing enzyme system in the little skate. *Bull. Mount Desert Island Bio. Lab.* 12: 12-15, 1972.

Bend, J. R., Pohl, R. J., and Fouts, J. R.: Further studies of the microsomal mixed-function oxidase system of the little skate, Raja erinacea, including its response to some xenobiotics. Bull. Mount Desert Island Biol. Lab. 13: 1973 (in press).

Pohl, R. J., Bend, J. R., Devereux, T. R., and Fouts, J. R.: Hepatic chemical and drug-metabolizing enzymes in coastal marine species. Bull. Mount Desert Island Biol. Lab. 13: 1973 (in press).

Bend, J. R., and Fouts, J. R.: Glutathione S-aryltransferase: Distribution in several marine species and partial characterization in hepatic soluble fractions from little skate (Raja erinacea) liver. Bull. Mount Desert Island Biol. Lab. 13: (in press).

Serial No.: NIEHS-P-007
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Environmental Contaminants: Uptake, Distribution, Excretion, and Sites of Storage in Rats and Selected Marine Species"

PREVIOUS SERIAL NUMBERS: NIEHS-PT-017; NIEHS-PT-035

PRINCIPAL INVESTIGATORS: J. R. Bend, Ph.D., L. G. Hart, Ph.D., M. O. James, Ph.D., and J. R. Fouts, Ph.D.

OTHER INVESTIGATORS: T. Devereux, M.S., G. L. Foureman, B.S., L. B. Gilmore, B.S., R. J. Pohl, B.S., D. P. Rall, M.D., Ph.D., and A. M. Guarino, Ph.D.

COOPERATING UNITS: Laboratory of Toxicology, National Cancer Institute; Environmental Biometry Branch, NIEHS

MAN YEARS:

Total:	1.2
Professional:	0.7
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are (1) to measure the uptake, distribution, metabolism and excretion of single, purified radiolabeled environmental contaminants, such as certain polychlorinated biphenyl (PCB) isomers, in selected marine species, and (2) to identify the subcellular site(s) of storage of lipophilic chlorinated environmental pollutants in liver and lungs of the rat.

METHODS EMPLOYED: At various times after administration (generally parenteral) of radiolabeled compound, specimens are sacrificed, plasma and tissues are removed, and aliquots are oxidized and/or solubilized prior to liquid scintillation counting. Urinary and biliary excretion rates are also monitored. Subcellular storage sites will be identified by radioautography on contract.

MAJOR FINDINGS:

1. The radioactivity associated with a single, intravascular (pericardial sinus) injection of 2,4,5,2',5'-pentachlorobiphenyl (^{14}C-PCB; 0.2 mg/kg) into the lobster (Homarus americanus) is very persistent, with most of the activity

residing in the hepatopancreas (over 90% of the total recovered radioactivity at 24 hours, 1 week, 2 weeks, 4 weeks and 8 weeks after treatment). The half-life of radioactivity in various tissues was estimated. Several tissues, including hepatopancreas ($t_{\frac{1}{2}}$ = 45 days), egg masses (39 days), stomach (24 days), gill (24 days), and intestine (23 days) demonstrated considerable retention of radiolabel, whereas others, such as plasma, green gland, heart, male gonad, tail muscle, and claw muscle all had half-lives of 5 days or less. Virtually all of the radioactivity remaining in hepatopancreas of lobsters sacrificed 2, 4 or 8 weeks after treatment was identified as unchanged 2,4,5,2',5'-pentachlorobiphenyl. Feces are the major site of elimination of ^{14}C-PCB in this species.

2. The distribution and disappearance from blood of ^{14}C-PCB was also investigated in female dogfish sharks (Squalus acanthias) following a single, intravascular injection (0.03 mg/kg). The liver was the major storage site at all times studied, and the specific activity (cpm/mg protein) in liver actually increased between 6 hours and 1 day after dosage. Only trace amounts of radioactivity were excreted in the urine. Bile contained more radioactivity than urine, but still accounted for an insignificant amount of the total dose. The disappearance of radioactivity from blood (after injection) follows a multiexponential decay with time and the initial rapid phase of the disappearance may correlate with uptake by liver. Most of the radioactivity in liver of dogfish (from 6 hours to 7 days after treatment) was identified as unchanged ^{14}C-PCB.

3. Virtually all of the radioactivity that is extracted from homogenates of rat liver or lungs at various times (5 min, 1 hr, 4 hr, 28 hr, 3 days and 5 days) after IV administration of ^{14}C-PCB occurs as unchanged ^{14}C-2,4,5,2',5'-pentachlorobiphenyl.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since compounds such as the PCBs occur widely in nature the storage, metabolism and elimination of these compounds in animals and man is of importance to environmental health. It is relevant to consider marine species since several pollutants are concentrated by the aquatic food chain and man relies upon several of these for a significant amount of foodstuff. Furthermore, the study of interaction between environmental contaminants, such as PCBs, and other xenobiotics including drugs, food additives, and pesticides with regard to competition for a common storage site in fish and other animals may aid in understanding increased toxicity of pollutant-drug, etc. mixtures.

PROPOSED COURSE: The subcellular storage site (e.g., the hepatic lipid droplet) for PCBs will be determined in rat liver and lung by radioautography. If this experimental approach is successful, similar investigations will be carried out with lobster hepatopancreas and dogfish liver.

The uptake, metabolism and elimination of major aliphatic and aromatic constituents of crude oil (petroleum) and of the herbicide 2,4-D will be studied in selected marine species.

PUBLICATIONS

Bend, J. R., Guarino, A. M., Briley, P. D., Rall, D. P., and Fouts, J. R.: Distribution of ^{14}C-2,4,5,2',5'-pentachlorobiphenyl in the lobster, Homarus americanus, after a single pericardial injection. Bull. Mount Desert Island Biol. Lab. 12: 9-12, 1972.

Bend, J. R., Bend, S. G., Guarino, A. M., Rall, D. P., and Fouts, J. R.: Distribution of ^{14}C-2,4,5,2',5'-pentachlorobiphenyl in the lobster, Homarus americanus, at various times after a single injection into the pericardial sinus. Bull. Mount Desert Island Biol. Lab. 13: 1973 (in press).

Hart, L. G., Fouts, J. R., and Bend, J. R.: Distribution and blood disappearance of ^{14}C-2,4,5,2',5'-pentachlorobiphenyl in the dogfish shark, Squalus acanthias, after intravascular administration. Bull. Mount Desert Island Biol. Lab. 13: 1973 (in press).

Serial No.: NIEHS-P-008
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Microsomal Mixed-Function Oxidase Systems: Specificity and Function"

PREVIOUS SERIAL NUMBER: NIEHS-PT-016

PRINCIPAL INVESTIGATOR: R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: J. R. Bend, Ph.D., and T. C. Orton, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	3.3
Professional:	1.8
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: The microsomal mixed-function oxidase (MFO) system of mammals exhibits species, sex, and tissue variations. Treatment of animals with various pharmaceutical agents, environmental pollutants, and other exogenous compounds can bring about changes in the MFO system(s). Both stimulation and inhibition of these systems can be elicited, and in some cases this can be accomplished with the same compound. It is not understood if these changes are due to direct effects on the major components of the MFO, to alterations in the membrane environment, or to changes in the concentrations or kinds of endogenous compounds that interact with the MFO.

The objective of this project is to increase our understanding of the factors responsible for the variances noted among mixed-function oxidase systems from different sources. This objective is being pursued via several approaches:

1. Tissue Differences: The MFO systems from rabbit liver and lung are being solubilized and separated into their component parts. These components (cytochrome P-450, TPNH cytochrome c reductase and lipid) and the enzymatic activities obtained by component recombination are being compared.

2. Species Differences: The hydroxylation of testosterone is being examined in liver microsomes from the adult rat and rabbit.

3. Differences in Responses to In Vivo Treatments: The effects of in vivo administration of 3-methylcholanthrene on the MFO in adult rat and rabbit liver are being studied.

METHODS EMPLOYED: The components of the liver MFO system are solubilized and separated by the methods developed by Lu and co-workers. These methods have been modified for application to the rabbit lung MFO system. Analysis of cytochrome b_5 and unreactive cytochrome P-450 in solubilized preparations is done by methods developed in this laboratory. The hydroxylation of testosterone is determined by thin layer chromatography. New methods allow for the quantification of the 2β-, 6β-, 7α- and 16α-hydroxytestosterone metabolites.

MAJOR FINDINGS:

1. The solubilized components of the rabbit lung MFO system exhibit different activity levels than those obtained from the liver. The lung cytochrome, which appears to be normal when examined spectrally, is significantly less active than the liver P-450 in support of benzphetamine N-demethylation and benzpyrene hydroxylation. This is the case when either the liver or the lung reductase fraction is utilized. The liver cytochrome is more active when combined with the lung reductase than when combined with the liver reductase. Addition of lung cytochrome to a system containing liver cytochrome and reductase results in inhibition of metabolic activity. A reductase preparation obtained from lung microsomes by sonication, detergent treatment and ammonium sulfate fractionation is active in metabolizing benzphetamine and 7-ethoxycoumarin without the addition of cytochrome fraction. This reductase preparation does not contain discernible levels of cytochrome P-450 but does contain significant cytochrome P-420.

2. The rates of hydroxylation of testosterone at the 2, 6, 7 and 16 positions differ in rat and rabbit hepatic microsomes. Effects elicited by in vivo (3-methylcholanthrene, phenobarbital) and in vitro (cytochrome c, benzphetamine, high ionic strength) treatments also differ markedly in the rat and rabbit. For example, phenobarbital treatment results in increased activities in the rat and inhibition in the rabbit. In addition, the effect of protein concentration and the Km's for the reactions are significantly different.

3. 3-Methylcholanthrene treatment of adult rats or rabbits results in the induction of hepatic, microsomal cytochrome P-448. In the rat several oxidative reactions, including the hydroxylation of benzpyrene, are also stimulated by 3-MC treatment. In the rabbit, however, these reactions are inhibited by similar treatment. The difference spectra of cytochrome P-448 from the rat and rabbit are essentially identical. However, examination of the absolute spectra shows that they may be significantly different. The oxidized spectrum of P-448 from the rabbit shows the presence of a type I substrate complex. This may result from the binding of 3-MC or a metabolite of 3-MC or some other ligand (exogenous or endogenous). Benzpyrene hydroxylase activity in a reconstituted system using cytochrome P-448

from rat liver microsomes is 10 times greater than that obtained using cytochrome P-450 from rat or rabbit or cytochrome P-448 from the rabbit.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of mixed-function oxidase systems is required if drug interaction, drug metabolism, disposition of ingested or inhaled environment pollutants, and pesticide detoxification are to be fully comprehended.

This problem is complicated by the multitude of functional differences and responses exhibited by MFO systems from various sources. Identification of the factors which control the activities of MFO systems is essential to our understanding and manipulation of this complex biological process.

PROPOSED COURSE: The present work involving the solubilization, reconstitution and determination of substrate specificities of the MFO systems from rabbit liver and lung will be continued. The effect of 3-methylcholanthrene in rat and rabbit will be further examined in an effort to identify the factors responsible for the differences noted in responses.

PUBLICATIONS

Orton, T. C. and Philpot, R. M.: Testosterone metabolism in rabbit liver and lung microsomes. Federation Proceedings 32, 701 Abs., 1973.

Orton, T. C. and Philpot, R. M.: Thin-layer chromatographic analysis of the metabolism of testosterone to 2β-, 6β-, 7α- and 16α-hydroxytestosterone by rabbit and rat hepatic microsomes. Anal. Biochem., accepted for publication, 1974.

Hook, G. E. R., Bend, J. R., Hoel, D., Fouts, J. R., and Gram, T. E.: Preparation of lung microsomes and a comparison of the distribution of enzymes between subcellular fractions of rabbit lung and liver. J. Pharmacol. Exptl. 182: 474-490, 1972.

Bend, J. R., Hook, G. E. R., Easterling, R. E., Gram, T. E., and Fouts, J. R.: A comparative study of the hepatic and pulmonary microsomal mixed-function oxidase systems in the rabbit. J. Pharmacol. Exptl. Ther. 183: 206-217, 1972.

Serial No.: NIEHS-P-009
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Interaction of Water-Soluble Substrates with Water-Insoluble Enzymes in Aqueous Media"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: G. E. R. Hook, Ph.D., J. R. Bend, Ph.D., and J. R. Fouts, Ph.D.

OTHER INVESTIGATORS: R. E. Easterling, L. B. Gilmore, B. A., and W. D. Taylor

COOPERATING UNITS: None

MAN YEARS:

Total:	1.2
Professional:	0.5
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: Most foreign compounds which are acted on by the microsomal mixed-function oxidase system are soluble to only a limited extent in water at physiological pH. The interactions of water-insoluble substrates with these enzymes have been studied extensively *in vitro*, but generally only with the aid of solubilizing agents designed to bring the substrate into aqueous solution. Often the effects of the solubilizing agent on the enzyme system under study have been completely ignored. The initial objectives of this study were to (1) investigate the effects of a number of currently popular solubilizing and suspending agents on liver microsomal electron transport as related to the hydroxylation of biphenyl, (2) if possible find a solubilizing or suspending agent which was itself a noneffector of microsomal electron transport, and (3) investigate the interaction of water-insoluble substrates with microsomes structurally modified by such membrane modifiers as detergents.

METHODS EMPLOYED: Microsomes were isolated from rabbit livers. Various aspects of microsomal electron transport were investigated using spectrophotometry and spectrophotofluorometry.

MAJOR FINDINGS: The organic solvents ethanol and dimethylsulfoxide inhibited biphenyl 4-hydroxylase and NADPH-cytochrome P-450 reductase. Acetone inhibited the hydroxylase uncompetitively at concentrations which appeared to stimulate NADPH-cytochrome P-450 reductase. The nonionic detergents Tween 20, Tween 80 and Triton X-100 inhibited biphenyl 4-hydroxylase although only Triton X-100 markedly affected the reduction of cytochrome P-450 by NADPH. Biphenyl caused a 2-3 fold stimulation of NADPH-cytochrome P-450 reductase, but in the presence of Tween 80 the stimulation was absent. Because Vmax of biphenyl 4-hydroxylase in the presence of Tween 80 was not significantly different from Vmax in its absence, it would appear that the reduction of cytochrome P-450 was not rate-limiting.

Of all the carriers studied, only carboxymethyl cellulose (CMC) was without effect on all aspects of microsomal electron transport investigated. As far as biphenyl 4-hydroxylase is concerned, CMC appears to be the most suitable substrate carrier.

The ability of the nonionic detergents Tween 20, Tween 80 and Triton X-100 to solubilize microsomal membranes and convert cytochrome P-450 into P-420 differs considerably. The P-450 to P-420 conversion rates in the presence of these detergents was greatly affected by the presence of biphenyl; with Triton X-100 the rate was increased and Tween 80 the rate was decreased but with Tween 20 no effect was seen.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Detergents are tools which may be used for the selective dismantling of biological membranes. The nonionic detergents studied differ considerably in their interactions with microsomal membranes and the chemical or drug-metabolizing enzyme system. The specific interactions of detergents with the microsomal membrane may provide considerable information concerning mechanisms of stimulation and inhibition of, and the nature of the chemical- and drug-metabolizing enzyme system as it exists in the microsomal membranes.

Knowledge concerning the nature of stimulation and inhibition of the chemical- or drug-metabolizing enzyme system is valuable since the interaction of man with his environment is to a large extent regulated by his ability to overcome its toxic components by processes such as metabolism of these components.

PROPOSED COURSE: Detergent interactions with microsomal components will be pursued. Studies of biphenyl metabolism and factors affecting it will continue both in terms of a general model system and as a prototype of the class of pollutants, PCB's.

PUBLICATIONS

Hook, G. E. R., Bend, J. R. and Fouts, J. R.: The effect of some biphenyl solubilizing and suspending agents on biphenyl 4-hydroxylase of rabbit liver microsomes. Chem-Biol. Interactions 7: 205-222 (1973).

Serial No.: NIEHS-P-010
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Studies on the Mechanisms of Accumulation of Drugs and Pollutants in the Lung: Investigation of the Transport of Substances from Pulmonary Circulation and Inhaled Air into Lung Tissue and of Metabolism of Xenobiotics by the Isolated Perfused Lung. Use of the Isolated Perfused Lung to Examine the Effect of Pollutants on Lung Functions"

PREVIOUS SERIAL NUMBER: NIEHS-PT-029

PRINCIPAL INVESTIGATORS: T. E. Eling, Ph.D., M. W. Anderson, Ph.D. (Environmental Biometry Branch), T. C. Orton, Ph.D., R. D. Pickett, Ph.D., F. Law, Ph.D., and J. R. Bend, Ph.D.

OTHER INVESTIGATORS: J. Carver, M.A.T. (Environmental Biometry Branch), J. Guthrie, B.S., and D. Parkes, B.S.

COOPERATING UNITS: Environmental Biometry Branch, Environmental Biology and Chemistry Branch (Histology, Electron Microscopy)

MAN YEARS:

Total:	4.0
Professional:	3.7
Other:	0.3

PROJECT DESCRIPTION

PART I: "Studies on the Mechanisms of Accumulation and Metabolism of Chemicals and Pollutants by the Lung"

OBJECTIVES: Study the processes and mechanisms for uptake and accumulation of basic chemicals in lung tissue. Assess the role of the lung in the disposition of chemicals and develop pharmacokinetic models for disposition of chemicals in the lung.

METHODS EMPLOYED: The lung perfusion apparatus consists of an artificial thorax, a recirculating blood supply, and an air supply. The lungs are ventilated with 95% air - 5% CO_2 by applying an alternating negative pressure to the artificial thorax. The circulatory system was designed with a blood reservoir directly above the lungs to which the labeled chemicals were added and from which samples of blood were removed for analysis. The apparatus was

modified (see Part III) so that the initial rates of uptake and the rates of efflux of chemicals from lung could be examined.

Metabolites of the compounds as found in the blood and lung are studied by thin-layer and paper chromatographic techniques. Viability of the perfused lung was confirmed by light and electron microscopy. Blood pH was also monitored over the perfusion period.

MAJOR FINDINGS: The ability of the isolated perfused lung (IPL) to metabolize several drugs and chemicals was investigated. The drugs, methadone and pentobarbital, and the pesticide, parathion, were rapidly degraded by the IPL, while imipramine and chlorcyclizine were not metabolized. However, all of these chemicals were degraded by fortified whole homogenates of lung. Thus metabolism of a chemical by lung homogenate cannot be completely extrapolated to the intact lung.

We have developed detailed models for the dynamic interaction between the perfused lung and circulating imipramine and amphetamine. For imipramine, the rate-limiting step for each of the two components of uptake is either binding or diffusion. Imipramine is accumulated in the lung by several types of binding sites. Some imipramine is translocated from one of these sites to be either irreversibly or very tightly bound since it was not released from the lung during an efflux study. The existence of this noneffluxable component of accumulated imipramine could have toxicological significance. Amphetamine is primarily accumulated in the perfused lung by a transport system, probably the norepinephrine transport system of the lung. All of the accumulated amphetamine can be effluxed from the lung. Thus, for these two basic amines, the lung employs different mechanisms in modulating their blood concentrations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a method for the perfusion of an isolated perfused lung allows studies with the lung uncomplicated by other organ functions. This preparation is perfused and ventilated similarly to an intact organ and thus correlation of *in vivo* and *in vitro* studies is likely.

The lung can act as an important site for accumulation of xenobiotics and this accumulation could result in detoxification or altered toxicity. In addition, those xenobiotics which accumulate in the lung could affect the accumulation and storage of other exogenous compounds or of endogenous substances such as norepinephrine and 5-hydroxytryptamine. The elucidation of the mechanism of accumulation could afford a means for predicting possible toxic effect and interactions of one chemical with another.

PROPOSED COURSE:

1. Further examine, through initial velocity and efflux studies, the mechanism(s) involved in the uptake and accumulation of amines and pollutants by lung.

2. Investigate in more detail effects of the nonnitrogenous portion of amines on uptake by the lung.

PART III: "Use of the Isolated Perfused Lung in the Study of the Pharmacological and Toxicological Effects of Environmental Agents, Administered via the Perfusate or by Inhalation, on Pulmonary Mechanics and other Lung Functions"

OBJECTIVES:

1. Establish suitable methods for use of the isolated perfused rabbit lung in pharmacokinetic, metabolic, hemodynamics and pulmonary mechanical studies.

2. Study the effects on respiratory mechanics, pulmonary hemodynamics, and nonrespiratory lung functions of environmental agents administered via the perfusate or by inhalation.

METHODS EMPLOYED: The apparatus and techniques are similar to those described in Part I.

MAJOR FINDINGS: The apparatus was modified by the addition of a number of recording devices for the measurement of perfusate flow rates, transpulmonary pressure, airway flow rates and inspiratory and expiratory volumes. From the recorded parameters, values can be calculated for certain mechanical properties of the lung, e.g., dynamic pulmonary compliance, airway resistance, time constant and total pulmonary vascular resistance. Perfusate characteristics are studied before, during and after perfusion, e.g., pCO_2, pO_2, hematocrit, etc.

Mechanical properties of the IPL were compared to respiratory mechanical measurements made in intact animals. The apparatus was also modified for studies of initial rates of influx and rates of efflux of chemicals from the lung. An infusion apparatus was connected to the pulmonary circulation to permit infusion of a chemical at a constant concentration regardless of perfusion rate. The infusion could be stopped during the experiment and the efflux of the accumulated chemical could then be examined. The apparatus was used in Parts I and II for studies of mechanism of amine accumulation in lung.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. To date, the isolated, blood-perfused rabbit lung has been used to study the uptake and accumulation of chemicals from the blood into the lung. In order to be able to interpret the results of such experiments, it is essential to know the physiologic status of the lung at any given point in the perfusion process. Because the ultimate goal is to extrapolate results obtained in the isolated perfused lung to the intact animal, a comparison of the *in vitro* and *in vivo* situations is required. In this phase of the project, collaborative studies with Dr. Robert Drew are foreseen.

2. The responses of the isolated perfused rabbit lung will be characterized prior to a study of the effects of "environmental agents." A comparison of the effects of chemicals on the lung as obtained in the isolated perfused rabbit lung with those obtained in the intact animal will provide an indication of extrapolative reliability and may also suggest possible mechanisms of direct action of chemicals on the lung.

3. Study the interaction between endogenous and exogenous amines at their sites of lung uptake and release.

4. Develop pharmacokinetic models for the accumulation of amines in lung tissue.

5. Assess the role of lung phospholipid in the accumulation of basic amines.

PART II: "Studies on the Uptake and Metabolism of Endogenous Substances by the Lung"

OBJECTIVES: The uptake and metabolism of endogenous substances, such as 5-hydroxytryptamine, norepinephrine, prostaglandins and angiotensin by lung. Examine the interactions between the accumulation of endogenous and exogenous substances by lung and study the effect of inhaled environmental agents on such nonrespiratory functions of the lung.

METHODS EMPLOYED: See "Methods Employed" in Part I.

MAJOR FINDINGS: Serotonin (5-HT) is removed from the venous circulation in lung by a uni-directional transport system which is Na^+ dependent. 5-HT is metabolized by the intracellular monoamine oxidase to 5-hydroxyindole acetic acid and this metabolite escapes by diffusion back into the arterial circulation. We have shown that the components of this detoxification system for 5-HT can be separately inhibited. For example, we have inhibited the metabolism step without affecting the transport system by using the monoamine oxidase inhibitor, pargyline. The 5-HT which was transported into the cell returned unaltered to the circulation by diffusion. Conversely, imipramine inhibits the transport system without affecting the metabolism.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung is an important site for the detoxification of several endogenous compounds. Understanding the mechanism for these detoxifications could aid in understanding lung function and the effect of certain environmental insults on the lung.

Elucidation of the mechanism of lung uptake and metabolism of chemicals might allow prediction of possible tonic interactions between endogenous and exposure substances.

PROPOSED COURSE:

1. Examine the mechanism for pulmonary accumulation and metabolism of endogenous vasoactive amines, e.g., norepinephrine.

2. Elucidate the mechanism responsible for the pulmonary uptake and metabolism of prostaglandins.

3. The isolated perfused lung permits examination of several nonrespiratory lung functions which might serve as development of "early warning systems" for lung damage from environmental toxicants.

PROPOSED COURSE: Develop the IPL system so as to measure respiratory mechanics and investigate the effects of inhaled environmental agents on such mechanics.

PUBLICATIONS

Orton, T. C., Anderson, M. W., Pickett, R. D., Eling, T. E., and Fouts, J. R.: Xenobiotic accumulation and metabolism by isolated perfused rabbit lungs. J. Pharmacol. Exp. Ther., Vol. 186, No. 3 (1973).

Pickett, R. D., Orton, T. C., Anderson, M. W., and Eling, T. E.: Accumulation of amines in the isolated, blood-perfused rabbit lung. The Pharmacologist, Vol. 15, No. 2 (1973).

Law, F. C. P., Bend, J. R., Eling, T. E., and Fouts, J. R.: Studies on the metabolism of drugs in the isolated perfused lung: The Pharmacologist, Vol. 15, No. 2 (1973).

Anderson, M. W., Orton, T. C., Pickett, R. D., and Eling, T. C.: Accumulation of amines in the isolated perfused rabbit lung. J. Pharmacol. Exp. Ther., 1974 (in press).

Eling, T. E., Pickett, R. D., Orton, T. C., and Anderson, M. W.: Studies on the transport of chemicals into lungs. Federation Procs., Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 1974.

Serial No.: NIEHS-P-011
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30 1974

PROJECT TITLE: "Binding of Basic Amines to Membrane Fractions from Rabbit Lung"

PREVIOUS SERIAL NUMBER: NIEHS-PT-030

PRINCIPAL INVESTIGATOR: R. M. Philpot, Ph.D.

OTHER INVESTIGATORS: Sarah Wilkinson, Bruce Woodin

COOPERATING UNITS: Pharmacokinetics Section, Pharmacology Branch

MAN YEARS:

Total:	0.8
Professional:	0.3
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: Basic amines are removed from the blood by the lung with varying degrees of efficiency. This process can be demonstrated in the whole animal and in isolated-perfused lung. Little is known about the subcellular localization of these accumulated compounds and the nature of the binding site(s) have not been determined. This project was initiated in an attempt to deal with these problems. The immediate objectives are:

1. To isolate lung subcellular fractions which are kinetically similar to the isolated-perfused lung in their ability to bind basic amines.

2. To determine the cellular origin of active constituents of lung fractions which bind basic amines.

3. To determine the composition of specific binding sites.

METHODS EMPLOYED: Basic amine binding to lung fractions is being studied using differential centrifugation and equilibrium dialysis. Quantification of binding is accomplished with ^{14}C amines and liquid scintillation techniques. Lung fractions are subdivided by digitonin binding and sucrose centrifugation.

MAJOR FINDINGS: The 100,000 x g pellet obtained from lungs perfused with ^{14}C imipramine contains the largest amount of bound drug. Steady state analysis of imipramine binding to this fraction gave the same binding constants obtained using the isolated perfused lung. Separation of plasma membrane and endoplasmic reticulum in the 100,000 x g fraction by digitonin treatment and sucrose density centrifugation indicates that imipramine binding is not associated with either of these cellular constituents. Radioactive profiles of bound imipramine obtained from sucrose density centrifugation of the 100,000 x g pellet show two distinct areas of binding. Bound imipramine is seen at sucrose densities less than those where endoplasmic reticulum or plasma membrane markers are observed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The binding of basic amines by the lung is important with respect to chemical and drug disposition and interaction in the lung. In order to fully understand the significance of this process it is necessary to determine the specific locations of chemical and drug binding, the mechanisms of transport, and the nature of any binding that may take place in the lung.

PROPOSED COURSE: Further work will concentrate on fractions from sucrose density centrifugation that contain bound imipramine. These fractions will be assayed for phospholipid, protein and membrane markers. Solubilization of these fractions will be carried out with subsequent chromatographic analysis to determine if the drug is protein bound and to study the nature of any binding seen.

Serial No.: NIEHS-P-012
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Uptake, Metabolism and Disposition of Aldrin by the Isolated Perfused Rabbit Lung"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATORS: E. A. El-Bassiouni, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.7
Professional:	0.7
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To study uptake, metabolism, accumulation and release of aldrin by isolated perfused lung preparations. To examine the mechanisms of uptake and the extent and the effect of aldrin metabolism on its disposition by the lung. To define the role of binding on aldrin uptake and release by the lung. The study is generally directed at understanding the fundamental mechanisms involved in the interaction of chlorinated environmental agents with vital physiological and biochemical systems in lung.

METHODS EMPLOYED: Rabbit lungs were surgically removed and maintained in a perfusion apparatus. An artificial medium was used as perfusate. Aldrin was introduced into the perfusate reservoir in 20 µl of ethanol after the lung had equilibrated for 15-20 minutes. Samples of the perfusate were taken at predetermined time points. Experiments were terminated after 60 minutes and the lung homogenized. Samples of lung homogenate and perfusate were extracted and analyzed for aldrin and its metabolite, dieldrin, using gas chromatography.

MAJOR FINDINGS: Analysis of the disappearance of aldrin from the medium indicated a biexponential decay corresponding to an initial rapid phase of uptake. Aldrin was metabolized by the lung to its epoxide, dieldrin, which was detected in the medium as early as three minutes after aldrin was added to the medium. During the second slower phase of uptake, aldrin in the medium was replaced by an equimolar amount of dieldrin (formed from aldrin by the perfused lung). Examination of total dieldrin produced at increasing substrate levels showed

a linear relationship up to 1 μmole, a curvilinear relationship from 1 to 2 μmoles, and a plateau beyond 2 μmoles. In addition, the half life of aldrin during the second phase of uptake remained constant up to 1 μmole aldrin, and increased beyond this point. These observations indicated that the slower phase of aldrin uptake was due to its metabolism by the lung to dieldrin. A linear relationship was obtained when aldrin in the lung at the end of 60 minutes was plotted against its concentration in the medium at that time. Hence, the accumulative processes are likely to be simple diffusion and non-specific binding.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aldrin, a chlorinated compound used extensively in the past as pesticide spray, was utilized in this study to gain some insight into the interaction of this class of xenobiotics with the lung. Lung metabolizes aldrin to its epoxide dieldrin and no further metabolism was detected. There is a considerable interest in the interaction of epoxides in general with various physiological systems. Several polycyclic hydrocarbons, for instance, are oxidized to an unstable epoxide and this epoxidation is thought to be related to carcinogenesis by these hydrocarbons. Pharmacokinetic studies with both aldrin and dieldrin will be carried out in order to understand the nature and the mechanisms of interaction of lung with these xenobiotics.

PROPOSED COURSE: Studies on the initial velocity of uptake will be carried out in order to more clearly define the initial rapid phase of uptake of aldrin by the lung. The extent and role of binding in aldrin accumulation by lung will also be investigated.

Serial No.: NIEHS-P-013
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Effect of Piperonyl Butoxide Aerosol Exposure on Uptake, Accumulation and Release of Amines by the Isolated Perfused Rabbit Lung Preparation"

PREVIOUS SERIAL NUMBER: NONE

PRINCIPAL INVESTIGATOR: H. M. Mehendale, Ph.D.

OTHER INVESTIGATOR: E. A. El-Bassiouni, Ph.D.

COOPERATING UNITS: Aerotoxicology Section, Pharmacology Branch

MAN YEARS:

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To investigate the effect of piperonyl butoxide (PB) aerosol by nasal exposure on lung uptake of amines such as imipramine (exogenous) and 5-hydroxytryptamine (endogenous). Preliminary experiments indicate that there is an increase in uptake of imipramine by lung from animals preexposed to PB aerosol. Investigations will be directed at explaining why such a change occurs; is it specific to imipramine, and is there a change in the metabolism of these compounds by the treated lungs.

METHODS EMPLOYED: Male rabbits were nasally exposed to PB aerosol at 8 ppm for 30 minutes and retained in their cages for 24 hours after exposure. Controls were subjected to similar treatment without PB. Lungs were surgically removed and perfused using an artificial perfusate. ^{14}C-Imipramine was added to the medium and samples of the perfusate taken at various time intervals. Experiments were terminated after 60 minutes. Perfusate samples were radio assayed.

MAJOR FINDINGS: Results indicate that 24 hours after animal preexposure to PB aerosol, imipramine uptake by the lungs from such treated animals increased by ≈ 58% over that in lungs from the untreated controls.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Piperonyl butoxide is most commonly used as a pesticide synergist and is present in many household spray cans of pesticides and is widely used in both industrial and farm type pest control programs. Effects of breathing PB on lung physiology and biochemistry as well as interactions of PB with other chemicals and drugs in the lung are almost unknown at this time.

PROPOSED COURSE: Experiments are being conducted to characterize PB-imipramine interactions in the lung - e.g. - dose-response, time-response relationships. Future investigations will be directed at understanding whether this change in uptake (caused by PB) is limited to imipramine, whether it involves changes in the metabolic ability of the lung, and whether it affects uptake, storage and release of endogenous amines such as 5-HT.

Serial No.: NIEHS-P-014
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Pharmacokinetics of Chlorinated Xenobiotics"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: M. W. Anderson, Ph.D. (Environmental Biometry Branch), M. Fields, K. Moore

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS:

Total:	2.0
Professional:	0.9
Other:	1.1

PROJECT DESCRIPTION

OBJECTIVES: To develop a pharmacokinetic model which will predict, for a number of species, the distribution, accumulation and excretion of a given chlorinated xenobiotic after different dose levels and routes of exposure.

METHODS EMPLOYED: The distribution and excretion of various ^{14}C-labeled polychlorinated biphenyl isomers were studied after IV administration to rats. The major tissues and excreta were sampled at times varying from a few minutes to several weeks post-treatment. Analyses were facilitated by the use of a biological material oxidizer and liquid-scintillation counting. The data from the analyses were further analyzed by a computer.

MAJOR FINDINGS: The initial studies were conducted on 2,4,5,2',5'-pentachlorobiphenyl (PCB). This compound was found to be initially stored in the muscle and liver and subsequently translocated to the fat and skin. The rate of PCB efflux from the fat and skin stores was much slower than in other tissues. The decay curves for disappearance of PCB from the blood and tissues were found to have three components. The major route of excretion from the body was through the bile and subsequently in the feces with a minimum of enterohepatic cycling. Most of the dose was excreted in the first few days after treatment; however, the remaining 15-20% of the dose was retained for several weeks. Less than 7% of the total dose was excreted in the urine. There was no apparent dose dependent effect on PCB distribution in tissues over the 0.6-6.0 mg/kg range. Studies with 4,4'-dichlorobiphenyl indicated that its distribution was similar

to that of pentachlorobiphenyl except that the time scale was significantly shortened, and excretion of dichlorobiphenyl in the urine is approximately five times greater than for pentachlorobiphenyl.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man.

PROPOSED COURSE: We plan to study the distribution and excretion of additional polychlorinated biphenyl isomers in the rat, and we plan to do distribution and excretion studies in other species. Ultimately we hope to use this data to construct a mathematical model for disposition of the polychlorinated biphenyls which can be extrapolated to other chlorinated xenobiotics and will predict the distribution and excretion of these compounds in a number of species.

PUBLICATIONS

Matthews, H. B., Bend, J. R., and Anderson, M. W.: Distribution and kinetics of 2,4,5,2',5'-pentachlorobiphenyl (PCB) in the male rat. Society of Toxicology, 13th Annual Meeting, Washington, D.C., March 1974 (in press).

Matthews, H. B., Chen, P. R., and Anderson, M. W.: The metabolism, storage and excretion of highly chlorinated compounds by mammals. American Chemical Society Symposium on Mechanism of Pesticide Action: Cellular and model systems. Los Angeles, California, March 1974 (in press).

Serial No.: NIEHS-P-015
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Transport of Xenobiotics by Blood and Its Constitutents"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: H. B. Matthews, Ph.D.

OTHER INVESTIGATORS: M. W. Anderson, Ph.D., and J. R. Surles, B.S. (Environmental Biometry Branch), J. G. Carver, B.S., M. Fields, and K. Moore

COOPERATING UNITS: Environmental Biometry Branch

MAN YEARS:

Total:	0.8
Professional:	0.3
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The blood is responsible for the transport of many endogenous substances throughout the body. It also carries either free or bound to its constituents most of the xenobiotics to which the organism is exposed. These studies were designed to first determine the mechanism of xenobiotic transport and secondly to determine if this transport in any way interferes with the normal transport of endogenous materials.

METHODS EMPLOYED: Radioactive (^{14}C) 2,4,5,2',5'-pentachlorobiphenyl was injected, intravenously, into rats and its distribution in various blood fractions was followed with time by the use of a biological material oxidizer, liquid scintillation counting, column and molecular sieve chromatography, and density gradient centrifugation. The distribution of other xenobiotics among various blood fractions, *in vitro*, was measured by the use of many of these same techniques. Chemicals studied were dieldrin, p,p'-DDT, hexachlorobenzene, carbaryl and testosterone.

MAJOR FINDINGS: After intravenous injection into rats, more than 95% of a total dose of pentachlorobiphenyl (PCB) disappeared from the blood within 5 minutes. Initially, approximately 60% of the dose was associated with the red blood cells and the remaining 40% was evenly distributed between the albumin and lipoprotein fractions of the plasma. Red blood cells released their associated PCB most rapidly followed by the lipoproteins. After

10 minutes most of the PCB remaining in the blood was associated with the albumin. By 8 hours after administration approximately 80% of the PCB in blood was associated with the albumin. In vitro, the chlorinated hydrocarbons studied were found to be associated with the lipoprotein fraction of plasma and the non-chlorinated hydrocarbons were not.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental exposure to a large number of xenobiotics via air, water or food contamination is significant. These materials are transported by the blood to various detoxication, excretion or storage sites within the body. Thus, if the transport of any one of these materials should interfere with the normal transport of endogenous materials by the blood then it could result in an imbalance of homeostasis.

PROPOSED COURSE: Current plans call for studies of blood transport of additional xenobiotics in an effort to correlate chlorination with duration of stay in the blood. Studies will also be made of the effect of these exogenous compounds on the transport of endogenous compounds, particularly, steroid hormones. Further, by use of the isolated perfused liver preparation, we hope to examine the role of binding to blood proteins in the uptake kinetics and disposition of these compounds by the liver.

Serial No.: NIEHS-P-016
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolism of Polychlorinated Biphenyls"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: P. R. Chen, Ph.D., and H. B. Matthews, Ph.D.

OTHER INVESTIGATOR: L. T. Jao, Ph.D.

COOPERATING UNITS: None

MAN YEARS:

Total:	1.1
Professional:	1.1
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine the nature of the metabolites of various polychlorinated biphenyl (PCB) isomers and the importance of each metabolite to the elimination of the parent compound from the animal.

METHODS EMPLOYED: The studies have utilized ^{14}C-labeled PCB isomers, and their *in vivo* metabolism by the rat to obtain the necessary metabolites. The metabolites were isolated by various extraction techniques, purified by thin-layer and column chromatography and identified by mass spectral and nuclear magnetic resonance analysis.

MAJOR FINDINGS: 2,4,5,2',5'-Pentachlorobiphenyl has been the subject of the most intensive studies to date. The metabolites of this compound were excreted primarily in the bile and subsequently in the feces. Less than 5% of the original dose was excreted as the parent compound. The major metabolite has been identified as 3'-hydroxy pentachlorobiphenyl and two minor metabolites have been identified as 3',4'-dihydrodihydroxy-pentachlorobiphenyl and 3',4'-dihydroxy-pentachlorobiphenyl. Less than 7% of the total dose was excreted in the urine, all of this was in the form of a glucuronide conjugate of the major metabolite, 3'-hydroxy-pentachlorobiphenyl.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The polychlorinated biphenyls are ubiquitous environmental contaminants, and knowledge of their biotransformation and the identification of their metabolites is essential to our understanding of their importance as potential hazards to

man and his environment. We have evidence that epoxides are formed in the metabolism of PCBs. Epoxides have been implicated in a number of undesirable biological reactions including teratogenesis, carcinogenesis and tissue necrosis.

PROPOSED COURSE: Continue the study of the metabolism of additional PCB isomers in an effort to determine the importance of position and degree of chlorination on the rate and nature of metabolite formation.

PUBLICATIONS

Chen, P. R., and Matthews, H. B.: Metabolism and excretion of 2,4,5,2',5'-pentachlorobiphenyl in the male rat. Society of Toxicology, 13th Annual Meeting, Washington, D.C., March 1974 (in press).

Serial No.: NIEHS-P-017
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Disposition of 2,4,5,2',5'-Pentachlorobiphenyl (PCB) and Indocyanine Green (ICG) in Biliary Fistula Rats and in Isolated Perfused Rat Livers"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: S. W. Hwang, Ph.D., and L. G. Hart, Ph.D.

OTHER INVESTIGATOR: Michael Clemons

COOPERATING UNITS: None

MAN YEARS:

Total:	1.0
Professional:	0.9
Other:	0.1

PROJECT DESCRIPTION

OBJECTIVES: Polychlorinated biphenyls (PCBs) are water insoluble synthetic chemicals which have been widely used in a variety of industrial applications. PCBs are found in tissues of different animal species and have known toxic effect in animals and man. This investigation is part of the research program designed to study comprehensively the pharmacokinetics of disposition and metabolism of 2,4,5,2',5'-pentachlorobiphenyl (PCB) in the rat. The main concerns of this investigation are the uptake, biliary excretion and enterohepatic circulation of PCB in rat. In addition to the study in intact rats with bile fistulas, the isolated perfused rat liver preparation was also used to obtain more specific pharmacokinetic data. Since PCBs are very water insoluble compounds, the hepatic disposition of a water soluble model compound, indocyanine green (ICG), was also studied for purposes of comparison. Further, the role of the perfusion media on hepatic disposition of PCB and ICG was evaluated by comparing blood with other artificial or semi-artificial perfusates.

PART I: "Studies on the Intact Bile-Fistula Rat"

METHODS EMPLOYED:

1. Biliary excretion of PCB: Rats weighing about 300-320 g were anesthetized with pentobarbital. Through a mid-line abdominal incision, the renal

pedicles were ligated and the common bile duct was cannulated with PE 10 tubing. Bile was collected and weighed. ^{14}C-PCB was dissolved in Emulphor and injected into the femoral vein. Blood samples were obtained through terminal heart puncture and livers were excised for analysis.

2. Intestinal absorption of PCB and its metabolites: Several rats were injected intravenously with PCB, 0.6 mg/kg, and bile samples were collected and pooled. A recipient group of rats was fasted, anesthetized, and a small incision was made in the duodenum about 1 cm distal to the entrance of the common bile duct. The ileocecal junction was ligated and the bile duct was cannulated. Aliquots of pooled bile containing 2.5 x 10^{-2} μM of PCB and its metabolites were infused through the incision into the intestine, followed by ligation distal to the incision. In some experiments, 2.5 x 10^{-2} μM of pure PCB solution was infused. After a fixed time interval, the percentages of the initial radioactivity in the aliquot remaining in the intestine and appearing in the blood, liver and bile were determined.

3. Retrograde intrabiliary absorption of PCB and its metabolites: For retrograde intrabiliary injection, a device similar to that used by Peterson and Fujimoto (J. Pharmacol. 185: 250, 1973) was used. The bile duct of the rat was cannulated with PE 10 tubing and precisely measured amounts of either PCB dose solution or bile containing PCB and metabolites were injected retrogradely into the biliary tree. The volume of injected solution was less than the capacity of the biliary tree. After injection each drop of bile excreted was collected individually in counting vials and the time intervals between drops were recorded.

Measurement of radioactivity: Bile samples were counted directly in a water-miscible scintillation solvent. Blood, homogenized liver and intestine were combusted with a biological materials oxidizer and the resulting $^{14}CO_2$ was counted in a liquid scintillation counter.

MAJOR FINDINGS:

1. Biliary excretion of PCB: Rats with ligated renal pedicles were injected intravenously with 0.6 mg/kg of PCB. During the first 30 min after injection, 5.3% of the dose was excreted in the bile, with about 32% of a dose excreted in 6 hours. Taurocholate infusion increased bile flow rates but did not alter excretion rates for PCB. Lowering body temperature decreased both bile flow and excretion rates for PCB. The bile/plasma concentration ratios for PCB and metabolites were usually greater than 10, suggesting that active transport processes might be involved. Most of the PCB was excreted in the form of more polar metabolites and conjugates. At least 45% of the excreted PCB was in the form of glucuronide(s), as determined by incubation of samples with β-glucuronidase. The excretion of PCB was increased after pretreatment of rats with 3,4-benzpyrene, while there was decreased excretion after prior treatment with SKF-525-A. Neither agent altered bile flow rates.

2. Intestinal absorption of bile containing PCB and its metabolites: This study provided evidence for an enterohepatic circulation for PCB and its metabolites in the rat. Thirty two percent of the radioactivity in pooled bile

from donor rats injected with PCB was absorbed in 3 hours from the intestines of recipient rats, while 9% was found in the liver, and 5% of the initial radioactivity was excreted in the bile of the recipient rats.

3. Intrabiliary absorption of PCB: Retrograde biliary infusion of either pure ^{14}C-PCB in Emulphor solution or bile from a donor rat containing primarily ^{14}C-PCB metabolites resulted in significant absorption of radioactivity from the biliary tree. The more lipid-soluble PCB was absorbed to a greater extent, 70-75%, than the PCB metabolites, 40-50%. The data supports the possibility of reabsorption of PCB and its metabolites during the excretion process.

PART II: "Studies on the Isolated Perfused Rat Liver"

METHODS EMPLOYED: The perfusion of liver was performed in a Miller-type liver perfusion apparatus. Different perfusion media were used: (1) Krebs-Ringer carbonate buffer containing 5 mM glucose and 4.5% bovine serum albumin, (2) heparinized rat blood diluted three times with medium 1, (3) washed bovine erythrocytes diluted with medium 1 to give a hematocrit of 0.35, (4) heparinized whole rat blood. Male rats, ≈ 325 g, were used as the liver donor. After pentobarbital anesthesia, the bile duct and portal vein were cannulated. The inferior vena cava was tied below the liver and a cannula was inserted in the vena cava above the diaphragm. The isolated liver was then connected to the liver perfusion apparatus with the portal vein cannula. The perfusion medium was preheated to 38° C and gassed with O_2-CO_2 (95-5%). Perfusion pressure was kept constant at 18 cm. Flow rate of the medium through liver was measured with an extracorporeal flowmeter. Radioactivity of PCB in bile and media were measured as previously described. ICG was measured spectrophotometrically.

MAJOR FINDINGS:

1. Differences in bile flow and perfusate flow with different media: The completely synthetic medium gave the highest perfusate flow rate through the liver but resulted in the lowest initial bile flow rates among all the media used. Liver flow rates with whole blood were the lowest but the initial bile flow rate was the greatest. Further, the half time for decreases in bile flow was 150 min using whole blood as the medium but only 70 min when synthetic medium was used. The other media studied were intermediate with regard to these parameters. Infusions of sodium taurocholate increased bile flow rates, with the most prominent effects of the choleretic occurring when synthetic media was used for perfusion of the liver.

2. ICG excretion in the bile by the perfused rat liver: The perfused liver showed different capacities for biliary excretion of ICG in different perfusion media. With totally synthetic medium, only 8.4% of an ICG dose was excreted in the bile (150 min), compared with diluted rat blood (22% in 150 min), washed bovine erythrocytes (29% in 240 min) and whole rat blood (48% in 240 min). In 240 min, an intact bile fistula rat excreted 95% of a comparable dose of ICG. As might be expected, the bile excretion rates were inversely correlated with the half times for disappearance of ICG from the media. The half times ranged from 54 min in synthetic medium to 16 min in whole blood.

3. Biliary excretion of PCB by perfused liver and the rates of disappearance of PCB in different perfusates: Biliary excretion rates of PCB by perfused liver also were different when different perfusates were used. With a dose of 0.24 mg PCB per 100 ml perfusate, the cumulative biliary excretion of PCB in synthetic medium was 2.5% of dose at 150 min and PCB disappearance rate constant was 0.92×10^{-3} min^{-1}; in medium with bovine red cells, the cumulative excretion was 3.3% of dose at 150 min and 4.1% of dose at 240 min and PCB disappearance rate constant was 1.63×10^{-3} min^{-1}; in whole rat blood, the cumulative excretion was 5.1% of dose at 150 min and 7.9% of dose at 270 min and the PCB disappearance rate constant was 2.02×10^{-3} min^{-1}. Comparison of PCB clearances showed that intact rats injected with 0.6 mg/kg PCB had a plasma PCB clearance of 0.91 ml/min and the isolated liver system using whole blood perfusate had a PCB clearance of 0.918 ml/min.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies of PCB disposition in animal systems may help us to understand how this widely used class of chemicals may be hazardous to the health of humans and animals. PCB was found to accumulate in the liver and its rate of excretion into the bile was relatively slow and largely rate limited by the hepatic metabolism of the compound. The study may help us to understand why the liver is especially susceptible to toxic effects from the PCBs. The experimental results in this study also delineated some characteristics of hepatic uptake, biliary excretion and intestinal absorption of lipophilic and hydrophilic compounds. These results may contribute to clarify the difference in hepatic handling of compounds with different lipid solubilities. Some of the pharmacokinetic values obtained in this study may be useful in the construction of mathematical models for the pharmacokinetics of PCB disposition in the whole animal.

PROPOSED COURSE: The isolated liver perfusion system is a good tool for in depth studies of hepatic uptake, storage and in situ metabolism of compounds, so the course to be followed is as follows:

1. A study of the effect of various chemical and physical factors on the initial uptake rates and storage of ICG or PCB in the liver including,

(a) temperature of the medium
(b) protein content of the medium
(c) pH of the medium
(d) oxygen
(e) metabolic inhibitors or other agents
(f) pretreatment of liver donor with selected agents

2. A study of initial uptake velocities with different concentrations of ICG or PCB in the medium and, if possible, calculation of the Km and Vmax for uptake.

3. A study of the rate of PCB metabolism in the perfused liver system and identification of metabolites.

Serial No.: NIEHS-P-018
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Inhalation Toxicity of Compounds Common to Household Aerosols"

PREVIOUS SERIAL NUMBER: NIEHS-PT-023

PRINCIPAL INVESTIGATORS: R. T. Drew, Ph.D., and G. J. Taylor, Ph.D.

OTHER INVESTIGATORS: J. R. Lo, Ph.D., B. H. Gupta, Ph.D. (Environmental Biology and Chemistry Branch), R. E. Staples, Ph.D. (Environmental Toxicology Branch), E. Lores, B.S., F. Harrington, M. T. Riley, J. Schroeder, and T. Clemmer (Environmental Biometry)

COOPERATING UNITS: Environmental Biology and Chemistry Branch (Pathology and Histology), Environmental Biometry Branch, Environmental Toxicology Branch

MAN YEARS:

Total:	4.1
Professional:	2.1
Other:	2.0

PROJECT DESCRIPTION

OBJECTIVES: To determine acute and chronic inhalation toxicity of selected compounds common to household aerosols and to identify any potential synergistic interactions. Compounds previously investigated include Rehydrol ASC (a propylene glycol complex of aluminum-chloride-hydroxide used in aerosol deodorants), "Airwick," hexachlorophene, and dichlorodifluoromethane (F-12). Compounds presently under investigation include Micro-dry (another aluminum-chloride-hydroxide compound not complexed with propylene glycol), a commercial preparation of hair spray, a commercial spray adhesive, F-12, and trichlorofluoromethane (F-11), and a series of other gases used as propellants or anesthetics.

METHODS EMPLOYED:

1. Exposure techniques: Exposures are carried out under dynamic conditions with contaminant concentrations being monitored frequently during exposures. Aerosols of aluminum-chloride-hydroxide are generated from an aqueous solution with a compressed air driven nebulizer. Commercial aerosols

are ejected into an elutriation chamber which traps the larger particles and then into an exposure chamber. The cans are activated mechanically on a 5-second on - 25-second off cycle. The fluorocarbons are blended with compressed air to the concentration desired and then directed to the chamber or in some cases to an endotracheal tube for direct exposure.

2. Toxicity evaluation: Several techniques are utilized to determine the effects of exposures. These include histologic preparation of tissue and microscopic examination for pathological or morphological changes; investigation of the mixed-function oxidase (MFO) activities of both lung and liver microsomes after animal exposure; assessment of weight gain and organ weights after exposure; investigation of any change in respiratory physiological parameters such as breathing rate and volume, resistance, and static and dynamic compliance; measurement of electrocardiographic and hemodynamic parameters, and myocardial contractility changes after and during exposures; and measurement of deposition and clearance of chemicals in the lung, and body distribution and elimination of chemicals after exposure.

MAJOR FINDINGS:

1. Aluminum: Exposure to either compound of aluminum for 3 days causes increases in lung weights in hamsters, rats and rabbits. This increase is maximal at 4 days and if exposures are stopped, the lung weight returns to normal in one week. The half life of aluminum in the lungs of exposed animals is about 40-50 days. Exposure of hamsters to a concentration of 50 mg/m^3 6 hr/day, 5 days/week for 6 weeks to either aluminum compound induces granulomatous lesions at the level of the peripheral bronchiole. These lesions persist up to 6 weeks postexposure, the longest time period investigated.

2. Adhesive sprays: Pregnant mice, rats and hamsters were exposed to the effluent from adhesive spray cans to investigate the teratologic potential of the adhesive sprays. A six hour exposure of female mice and rats at three different stages of pregnancy and of hamsters at one stage of pregnancy failed to produce any overt teratology.

3. Hair sprays: Hamsters were exposed 6 hr/day 5 days/week for 6 weeks to an aerosol hair spray in an intermittent fashion such that the actual spraying time over a 6 hour period was 12 minutes. The lungs from exposed animals were distinguishable from control lungs, but the changes were not remarkable and appeared to diminish after the exposure.

4. Fluorocarbons: Acute exposure of rabbits to F-12 results in a dose-related depression in left ventricular conduction and cardiac output without associated changes in heart rate, blood pressure, arterial PO_2, PCO_2 or pH. The evidence supports the conclusion that this is a direct effect of F-12 on myocardial contractility. Intermittent exposure to 10% F-12 (6 hr/day for 29 days) does not alter the response to an acute challenge of F-12, suggesting that cardiotoxic effects of F-12 are not cumulative. The response of a strain of hamsters with an inherited cardiomyopathic trait has been compared to the

response of normal hamsters to exposure to F-11. Myopathic hamsters developed arrythmias at lower F-11 concentrations than did random-bred hamsters and the character of the arrythmia produced was different in the myopathic hamster. Exposure to 7.5% F-11 killed all older (240 day) hamsters without any effects on younger (150 day) myopathic hamsters or age matched random-bred controls.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of the aerosol "spray can" dispenser has resulted in a very complex and wide variety of materials being available in respirable form. While the acute toxicity of many of the "active ingredients" in such dispensers has been investigated, little is known about the chronic toxicity of these compounds or the toxicity of combinations of these compounds and "inert ingredients" of the pressurized cans. Elucidation of the chronic toxicity of these compounds and their potential combinations can be of vital importance to environmental health.

PROPOSED COURSE: The studies on aluminum are being phased out. Studies on fluorocarbons will continue, assessing cardiovascular effects of inhalation of fluorocarbon and comparing the various fluorocarbons. Studies of possible interaction of fluorocarbons with other chemicals, i.e., effects on uptake, storage, retention and excretion are being considered. Other commercial products may be investigated.

PUBLICATIONS

Drew, R. T., Gupta, B. N., Bend, J. R., and Hook, G. E. R.: Inhalation studies with a glycol complex of aluminum-chloride-hydroxide. Arch. Environ. Hlth. (in press), 1974.

Taylor, G. J., and Drew, R. T.: Cardiovascular effects of chronic and acute fluorocarbon 12 exposures in rabbits. Presented at the Fourth Conference on Environmental Contamination at Wright-Patterson Air Force Base, October 1973 (Proceedings in press).

Drew, R. T., Lo, J. R., and Gupta, B. N.: The inhalation toxicity of aluminum-chloride-hydroxide. (Abs.) Presented at the American Industrial Hygiene Conference, Miami, Florida, May, 1974.

Serial No.: NIEHS-P-019
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Effects of Drugs and Chemicals on the Inhalation Toxicity of Chemical Solvents"

PREVIOUS SERIAL NUMBER: NIEHS-PT-003

PRINCIPAL INVESTIGATORS: R. T. Drew, Ph.D., and C. Harper, Ph.D.

OTHER INVESTIGATORS: B. Gupta, Ph.D., and J. Zinkl, Ph.D. (Environmental Biology and Chemistry Branch), J. R. Lo, Ph.D., R. P. Roth, Ph.D., M. Hogan, Ph.D. (Environmental Biometry), J. R. Fouts, Ph.D., F. W. Harrington, E. Jackson, E. M. Lores, B.S., M. T. Riley and J. L. Schroeder

COOPERATING UNITS: Environmental Biology and Chemistry Branch (Histology), Environmental Biometry Branch

MAN YEARS:

Total:	3.9
Professional:	2.1
Other:	1.8

PROJECT DESCRIPTION

OBJECTIVES: Liver is known to contain microsomal toxication and detoxication enzymes that are inducible by certain drugs and other chemicals. There are suggestions that analogous lung enzymes are also inducible. Detoxication of some organic solvents is catalyzed by some of these microsomal enzymes. This study has the following objectives:

1. Design a reliable method for measuring the enzymatic degradation of benzene by lung and liver microsomes.

2. Design a reliable method for measuring the enzymatic degradation of paraxylene by lung and liver microsomes.

3. Determine if pretreatment of animals with drugs and other chemicals which modify liver and/or lung microsomal enzymes will alter the acute and chronic inhalation toxicity of selected organic solvents.

4. Determine whether pretreatment (by inhalation) by common solvents affects their enzymatic degradation in lung and liver.

5. Study the species differences in benzene metabolism by rat, rabbit and hamster microsomal enzymes from both lung and liver. Define as many as possible of the biochemical parameters that affect benzene metabolism in both tissues of the three species.

6. Investigate some aspects of the biochemical conversion of dichloromethane to carbon monoxide.

7. Determine if inhalation exposure to known hepatic microsomal enzyme inducers affects analogous lung enzyme activities.

8. Identify the various metabolic pathways involved in the enzymatic degradation of paraxylene and determine whether pretreatment by known inducers stimulates different metabolic pathways.

9. Identify urinary metabolites for use as biological indicators of paraxylene exposure.

MAJOR FINDINGS:

1. A reliable, reproducible method has been developed to assay for benzene metabolism by lung and liver microsomes.

2. Animal pretreatment by known hepatic microsomal enzyme inducers increases metabolism of benzene by liver microsomes from both the rat and the rabbit while not affecting metabolism of benzene by lung microsomes. These enzyme inducers do not affect the acute toxicity of inhaled or injected benzene in rats.

3. Three 4-hour inhalation exposures to 4,000 ppm benzene doubles the rate of benzene metabolism in rat liver but not in lung. Three 4-hour inhalation exposures to 2,000 ppm benzene increases the rate of benzene metabolism in rat liver by about 10 percent.

4. Base-line rates of *in vitro* hepatic microsomal benzene metabolism are similar for male and female rats but animal pretreatment with phenobarbital will induce *in vitro* benzene metabolism in male rats to a greater extent than in females.

5. Chronic exposure of rats to benzene vapors (1,650 ppm, 6 hrs/day 5 days/week for 12 weeks) produces a depressed growth rate, a diminished lymphocyte count and a slight increase in the *in vitro* rate of hepatic microsomal metabolism of benzene. Continuous administration of phenobarbital in the drinking water of rats given the same benzene inhalation exposure regime partially reverses the diminished growth rate, partially protects against the lymphocytopenia and markedly increases the rate of hepatic microsomal metabolism of benzene.

6. A reliable method has been developed to assay paraxylene metabolism to toluic acid by lung and liver microsomes.

7. The effects of pretreatment of rats with phenobarbital, chlorpromazine or 3-methylcholanthrene on inhalation toxicity (LC_{50}), intraperitoneal toxicity (LD_{50}), and hepatic microsomal metabolism of paraxylene are shown below.

Treatment	LC_{50} ppm	LD_{50} mg/kg	nmoles metabolized/min/mg protein
Saline	4,700	3.8	0.31
Phenobarbital	5,800	3.6	1.03
Chlorpromazine	5,000	4.1	0.49
Corn Oil	4,600	3.8	0.36
3-Methylcholanthrene	5,000	4.8	1.53

8. Preliminary results indicate that microsomes from liver and lungs of rabbits are about 15 times more active than those from rats in metabolizing paraxylene to toluic acid.

9. The maximum increase in percent carboxyhemoglobin (% COHb) in the blood of rabbits after 20 minute inhalation exposure to dichloromethane (DCM) has been characterized as a function of the DCM concentration. Multiple exposures to DCM do not increase the percent COHb above that seen after only one exposure. Pretreatment of rabbits with SKF 525-A or pregnenolone 16α carbonitrile does not significantly alter the percent COHb resulting from exposure to DCM. However, pretreatment of rabbits with phenobarbital, an hepatic microsomal enzyme inducer and CCl_4, a potent hepatotoxin both markedly reduce the percent COHb resulting from exposure to DCM.

10. Inhalation exposure to phenobarbital aerosols induces hepatic microsomal metabolism of benzene but does not alter pulmonary microsomal metabolism.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In today's society the number of people taking drugs is substantial. Some of these compounds stimulate liver microsomal enzymes, and possibly lung systems, thus increasing the rate at which certain exogenous chemicals and/or endogenous substrates are metabolized. These studies are designed to better define the toxic responses to selected solvents and to determine if the toxicity of these solvents can be altered by pre-exposure to themselves or to other drugs. The effect of the route of widespread industrial and household use and because it is now being proposed for use instead of Tetraethyl lead in increasing gasoline octane ratings.

The recent findings that humans who are exposed to dichloromethane have elevated carboxyhemoglobin has generated considerable interest. Studies designed to elucidate the nature and site of this reaction have been started. Should this metabolism occur exclusively in lung, a model of pulmonary metabolism analogous to the hexabarbital sleeping time model for hepatic metabolism could be used to study effects of a variety of agents.

PROPOSED COURSE: Solvent toxicity and metabolism and the modification thereof by known inducers and inhibitors will continue to be investigated. Particular emphasis will be placed on identification of other metabolites from paraxylene and on the pathways of metabolism stimulated by certain inducers. An attempt will be made to identify urinary metabolites.

PUBLICATIONS

Drew, R. T., and Fouts, J. R.: The lack of effects of pretreatment by phenobarbital and chlorpromazine on the acute toxicity of benzene. Toxicol. Appl. Pharmacol. 27: 183-193, 1974.

Drew, R. T., Harper, C., and Fouts, J. R.: The influence of certain drugs on the metabolism and toxicity of benzene. Proceedings of the Symposium on Benzene and Alkyl Benzenes, Aug. 28-29, 1973, Mellon Institute, Pittsburgh, Pa., Industrial Health Foundation, pp. 17-31, 1974.

Roth, R. P., Lo, R. J., and Drew, R. T.: Dichloromethane inhalation and drug-metabolizing enzymes. Effects of pretreatment with inducing and inhibiting agents on carboxyhemoglobin levels. Presented at the Fourth Conference on Environmental Contamination at Wright-Patterson Air Force Base, October, 1973. (Proceedings in press)

Harper, C., Drew, R. T., and Fouts, J. R.: Benzene metabolism in mammalian tissue. (Abs.) Presented at the 1973 Fall ASPET Meeting, East Lansing, Michigan, August, 1973.

Drew, R. T., and Fouts, J. R.: The effects of inducers on paraxylene toxicity in rats. (Abs.) Presented at the Annual Meeting of the Society of Toxicology, March, 1974.

Drew, R. T., Harper, C., Zinkl, J., Gupta, B. N., and Hogan, M. D.: The effect of phenobarbital on chronic benzene toxicity in rats. (Abs.) Presented at the Annual Meeting of the Society of Toxicology, March, 1974.

Serial No.: NIEHS-P-020
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Influence of Inhaled Environmental Agents on Mammalian Pulmonary Mechanics"

PREVIOUS SERIAL NUMBER: NIEHS-PT-024

PRINCIPAL INVESTIGATORS: R. P. Roth, Ph.D., and R. T. Drew, Ph.D.

OTHER INVESTIGATORS: T. E. Eling, Ph.D., R. D. Pickett, Ph.D., F. Harrington, M. T. Riley

COOPERATING UNITS: Environmental Biology and Chemistry Branch

MAN YEARS:

Total:	0.8
Professional:	0.5
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: Pulmonary function studies will be conducted utilizing standard and newly-devised physiological techniques to assess the effects of chronic and acute inhalation exposures to irritants, oxidants, solvents, and propellants on the intact rabbit. These findings will be correlated with data from cardiovascular, biochemical and histological investigations. The respiratory physiologic data derived from these studies will be compared with similar data derived from the isolated perfused rabbit lung to assess the usefulness of such a test model.

MAJOR FINDINGS: Studies have concentrated on development of the methodology. Comparative results in terms of pulmonary resistance, dynamic compliance and time constants indicate reasonable agreement between *in vivo* and *in vitro* preparations.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our approach to studies of effects of environmental chemicals on this organ system will be to use sensitive pulmonary function indices such as frequency-dependent compliance and possibly critical closing volume to provide early detection of pathologic processes in the small airways. The ability to measure subtle changes in respiratory physiologic parameters may provide us with another tool to assess the effects of inhaled materials.

PROPOSED COURSE: This project will continue with emphasis in four general areas: (1) to assess the respiratory physiologic effects of single and multiple exposures to individual chemical compounds and combinations of compounds, (2) further characterization of normal physiologic parameters for comparison with *in vitro* studies, (3) comparison studies involving responses to known pharmacologic agents, and (4) comparison of respiratory physiological parameters *in vitro* in the isolated perfused lung with those measured *in vivo* in the intact rabbit.

Serial No.: NIEHS-P-021
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Composition and Origins of the Acellular Lining Layer of the Lung"

PREVIOUS SERIAL NUMBER: NIEHS-PT-028

PRINCIPAL INVESTIGATORS: G. E. R. Hook, Ph.D., and R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: L. B. Gilmore, B.A., and D. L. Eckberg, B.S.

COOPERATING UNITS: Environmental Biology and Chemistry Branch (Histology and Electron Microscopy)

MAN YEARS:

Total:	1.0
Professional:	0.4
Other:	0.6

PROJECT DESCRIPTION

OBJECTIVES: Numerous chemical analyses of the acellular lung lining layer have been made and there is little doubt concerning the identity of the surfactant material obligatory for normal lung function. However, the acellular layer is also a barrier to the environment and consequently may possess numerous, as yet unrealized properties. We are attempting to show that the medium bathing the alveoli is not merely required for its surface-tension-lowering properties but that it is also an actively metabolizing milieu.

The objectives of this investigation are (1) to determine the enzymatic and protein composition of the lung lining, (2) to determine the origin of these activities, and (3) to investigate compositional variations of the lining under the influence of drugs, disease and airborne pollutants.

METHODS EMPLOYED: The acellular lung lining is removed by lavage. The lavage is analyzed for cell, chemical and enzymic composition using microscopic, spectrophotometric, chromatographic and electrophoretic methods.

MAJOR FINDINGS: We have detected a number of enzymes and other protein components in the extracellular lung-lining layer. Two of the three major protein components which are not serum-derived appear to be glycoproteins. One of the glycoproteins seems to have a high affinity for the phospholipid

component of the extracellular layer and is also present in isolated and purified concentric lamellar organelles of the type II cell. Enzymic activities which we have detected in lung lavages appear to derive from three compartments which are related to (1) the acellular lining layer, (2) the capillary bed, and (3) cell damage. A number of lysosomal acid hydrolases appear to be associated with the acellular lining layer. The major enzyme component that we have detected in lung lavages is β-N-acetylglucosaminidase; approximately 5-10 percent of the total lung enzyme is found at the lining. The enzyme appears not to be serum-derived since the lung and serum enzymes differ electrophoretically on acrylamide gels. The β-N-acetylglucosaminidase of the lung lining seems to have a high affinity for the phospholipid component of the extracellular lining layer.

Both β-N-acetylglucosaminidase and one of the major glycoprotein components of the acellular lining layer may be selectively secreted by the type II cell.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Protein components of the acellular lung lining may play important roles in its regulation as well as defense of the lung. The origins of the acellular lung lining are in doubt and our studies provide strong evidence for an exocrinic function of the alveolar type II cell.

Of considerable importance are methods which can provide evidence of disease or damage to the human lung. If, as preliminary data suggests, three compartments can be distinguished as far as contributions to the lung lining are concerned, then it may be possible to assess to a certain extent the degree and type of damage which has occurred to the lung merely by assaying lavage fluids for enzymic activities. For example, enzymes associated with serum would tend to increase in the lavage in cases where damage has occurred to capillary walls. Such an enzymic assessment could serve in the diagnosis of respiratory disease using sputum or lavage samples obtained at the clinic.

PROPOSED COURSE: The properties of the protein components of the acellular layer will be investigated. The use of these components as diagnostic tools for lung disease in adult animals will be looked at as well as their possible use as markers of lung maturity in fetal animals.

Serial No.: NIEHS-P-022
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Biosynthesis and Degradation of Lung Surfactant Phospholipids"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATORS: G. Hook, Ph.D., T. Abe, Ph.D., D. Eckberg, B.S.

COOPERATING UNITS: Dr. W. B. Lynn and Assocs.
Department of Biochemistry
Duke University School of Medicine
Durham, N.C.

MAN YEARS:

Total:	2.1
Professional:	1.1
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The lung surfactant system is vital in maintaining alveolar stability. This stability is thought to be conferred, for the most part, by dipalmitoylphosphatidylcholine, which is secreted to the acellular alveolar lining layer of the lung. The overall objectives of this project are to investigate the cellular source of surfactant phospholipid in the rabbit lung. Special attention was given at the outset of this project to the isolation and study of the rabbit lung concentric laminar organelles (CLO) located in the type II cell of the lung epithelium.

METHODS EMPLOYED: The CLO from rabbit lungs were isolated using differential and sucrose-gradient density centrifugation. Electron microscopy confirmed that these structures could be isolated intact and without contamination by other cellular structures. Other findings are summarized below:

1. The CLO contained mostly phosphatidylcholine (PC) and phosphatidylethanolamine (PE), with negligible amounts of neutral lipid. Palmitic acid was the major fatty acid in these phospholipids. A phospholipid : protein ratio of 12:1 was observed and served as an index of purity.

2. The CLO consisted of numerous acid hydrolases similar to those observed in liver lysosomes. No phosphatidylcholine acyl hydrolase could be detected using [^{14}C-fatty acid]-labeled PC.

3. Prolonged (< 3 hrs) electrophoresis of the Triton X-100-solubilized CLO revealed the presence of a single prominent periodic acid-Schiff-positive material. In order for this material to penetrate gels, low polyacrylamide concentrations (4-5%) had to be used and Triton X-100 (0.1%, v/v) had to be mixed with the polyacrylamide. This unknown material could also be detected on gels by the use of several protein stains. This material has also been identified in a "surfactant fraction" obtained by trachea-bronchial lavage of rabbit lungs.

4. Injection of $NaH_2{}^{32}PO_4$ into rabbits revealed a rapid uptake of this isotope into the lung CLO. Pulse studies of up to 6 hrs showed that the specific activity (^{32}P cpm/μg PLP_P) remained much higher in the CLO than in surfactant (lung lavage) phospholipids. The major lipids labeled in the CLO and in the lung lavage $CHCl_3$:CH_3OH extract were PC and PE.

These results support a secretory or exocrine role for the lung type II cell and suggest that the CLO are the source of the major extracellular surfactant phospholipids and glycoproteins at the alveolar lining layer.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the participation of the CLO in the surfactant system of the lung would help clarify whether or not these structures are important as a rate-limiting step in the elaboration of surfactant. A number of gases and drugs have been reported to affect the number and size of the CLO present in the type II cells. The CLO are also currently being investigated at this Institute for their role in maintaining high concentrations of certain chemicals and drugs in the lung.

In infant respiratory distress syndrome, the CLO are essentially evacuolated while the lung is in atelectasis and in this disease the lung contains less surfactant phospholipids. This suggests a correlation between structure and function for the CLO.

The presence of a putative lung-specific glycoprotein might be the basis for a test for early detection of lung diseases such as emphysema.

PROPOSED COURSE: Studies of this exocrinic function of the type II cells are currently being written up for publication. Specific studies shall now be directed to the lung alveolar glycoprotein in attempts to:

(1) further purify and isolate this material in order to determine its compositional and physical properties.

(2) develop an antibody test for this compound.

(3) discern whether or not this glycoprotein is lung-specific or exists in other cells.

(4) prove or disprove that this glycoprotein is indeed secreted to the lining of the lung from an intracellular organelle such as the CLO. The perfused rabbit lung shall be used in these trials.

(5) reveal the presence of a similar glycoprotein in humans and to understand how the level or biosynthesis of this glycoprotein might be modified in "glycoprotein" disorders such as cystic fibrosis.

PUBLICATIONS

DiAugustine, R. P.: Lung concentric laminar organelle. Hydrolase activity and compositional analysis. J. Biol. Chem. 249: 584-593, 1974.

Bhattacharyya, S. N., DiAugustine, R. P., and Lynn, W. S.: Isolation and characterization of the major glycoproteins of rabbit lamellar bodies. Arch. Biochem. Biophys., in press, 1974.

Serial No.: NIEHS-P-023
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Prostaglandin Biosynthesis and Metabolism by Lungs. Effect of Pollutants on this System"

PREVIOUS SERIAL NUMBER: NIEHS-PT-022

PRINCIPAL INVESTIGATORS: T. E. Eling, Ph.D., and M. W. Anderson, Ph.D. (Environmental Biometry Branch)

OTHER INVESTIGATOR: D. G. Parkes, B.S.

COOPERATING UNITS: E. O. Oswald, Ph.D., and R. T. Drew, Ph.D.

MAN YEARS:

Total:	1.0
Professional:	0.3
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: This is a continuing project directed toward obtaining a better understanding of lung function. The primary objective is to investigate the biosynthesis and metabolism of prostaglandins by the lung and to relate changes in steady-state concentrations of the various prostaglandins to biological effects. In addition, the effects of various environmental agents (SO_2, O_3, NO_2, O_2, etc.) on the ability of the lung to synthesize and degrade prostaglandins will be investigated. An attempt will be made to correlate the biological effect of pollutants and modulation of prostaglandin levels.

METHODS EMPLOYED: Prostaglandins are analyzed by a combination of quantitative thin-layer chromatography and liquid scintillation counting. The radioactive prostaglandins, produced by lung tissue from the precursor ^{14}C-arachidonic acid, are extracted from the incubation medium and separated on thin-layer plates. The radioactive areas are measured by liquid scintillation techniques. Guinea pig lung serves as an enzyme and cofactor source for both biosynthesis and metabolism. Further identification of the prostaglandins uses a combination of thin-layer chromatography, continuous gradient elution column chromatography and mass spectrometry. Prostaglandin metabolism was estimated by incubation of ^{3}H-$PGF_{2\alpha}$ with the enzyme source, extraction from the incubation mixtures and separation of nonmetabolized ^{3}H-$PGF_{2\alpha}$ from metabolites. Metabolism was measured by the disappearance of ^{3}H-$PGF_{2\alpha}$.

MAJOR FINDINGS: Lung tissue biosynthesizes and degrades prostaglandins (PG). The PG synthesizing system in guinea pig lung converts ^{14}C-arachidonic acid to PGE_2, $PGF_{2\alpha}$ and an unknown PG-like material. An assay for the enzyme system was developed, characterized and optimized. We have established the chemical identity of PGE_2 and $PGF_{2\alpha}$ produced by our enzyme system with GLC-mass spectrometry. The unknown has been partially identified by mass-spectrometric techniques.

PG are metabolized by guinea pig lung dehydrogenases and reductases (at 13, 14 position). Using ^3H-$PGF_{2\alpha}$ as substrate, an *in vitro* assay system was developed. The metabolic products of ^3H-$PGF_{2\alpha}$ were isolated and identified by combined GLC-mass spectrometry.

The effect of environmental agents on the rates of biosynthesis and metabolism *in vitro* was investigated. Exposure of guinea pigs to various concentrations of SO_2 for various times, did not alter either PG biosynthesis or metabolism as measured *in vitro* in preparations from these exposed animals. Exposure of guinea pigs to 100 percent O_2 for 48 hours did not alter biosynthesis, lung weight, or lung total protein, but did depress PG metabolism from these animals. Metabolism of PG *in vitro* was depressed by 100 percent O_2 exposure of animals in a time-dependent manner and reached 90 percent inhibition after 72 hours of O_2 exposure. The effects of animal exposure to O_2 may be relatively specific since other soluble lung enzymes were not depressed by these exposures.

Exposure of guinea pigs to 90 ppm of NO_2 for 6 hours also depressed PG metabolism *in vitro*, thus suggesting a general toxic effect of oxidants on the metabolizing enzyme system. The effect of O_3 exposure of guinea pigs on guinea pig prostaglandins biosynthesis and metabolism *in vitro* is currently being investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Prostaglandins represent a new class of endogenous substance which possess unique biological activity in lung. Prostaglandins of the E class are potent bronchial dilators, while prostaglandins of the F class are potent constrictors. Both classes of prostaglandins have been implicated in inflammatory responses. Because these substances are not stored in most tissue, control of biosynthesis and metabolism is important to their physiological activity. Pathological changes or conditions produced in various disease states or in response to pollutants or environmental agents could involve an effect on either prostaglandin biosynthesis or metabolism. Understanding the mechanisms for the control of prostaglandin levels in lung tissues should contribute to the overall understanding of biological functions of these lipids.

Depression of the lung-metabolizing system could be of direct medical importance because prostaglandins and their derivatives have been used as therapeutic agents. Thus interactions are possible and should be studied.

PROPOSED COURSE:

1. Investigate the effect of animal exposure to oxidants (O_3, NO_2) on the PG-metabolizing system of guinea pig lung.

2. Establish time course and dose-response to oxidants (O_3, NO_2) on the PG-metabolism.

3. Study the mechanism of inhibition of metabolism. Determine if this inhibition is a specific effect related to oxidant toxicity to lung, or is a nonspecific effect.

4. Determine the pathologic and/or physiologic effects of increased PG in lung.

5. Investigate effects of respiratory diseases or allergic conditions on biosynthesis and metabolism of PG in lung.

6. Perform comparative metabolism studies using isolated perfused lung preparations (comparison with microsomal systems).

7. Investigate PG metabolism and oxidant effects on this metabolism in other animal species.

PUBLICATIONS

Eling, T., and Parkes, D.: Studies on biosynthesis of prostaglandins by guinea pig lung. (Abs.) Presented at ASPET Meeting, Aug. 19-23, 1973, East Lansing, Michigan.

Eling, T., and Parkes, D.: Studies on biosynthesis of prostaglandin by guinea pig lung. (Abs.) Presented at Ninth International Congress of Biochemistry, July 1-7, 1973, Stockholm.

Oswald, E., Parkes, D., Eling, T., and Corbett, J.: Characterization of prostaglandins by combined gas liquid chromatography and chemical ionization mass spectrometry. J. Chromatog., in press, 1974.

Parkes, D., and Eling, T.: Characterization of prostaglandin synthetase in guinea pig lung: Isolation of a new prostaglandin derivative from arachidonic acid. J. Biochem., in press, 1974.

Serial No.: NIEHS-P-024
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Lung Membrane-Bound Nicotinamide Adenine Dinucleotide (NAD) Glycohydrolase"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. P. DiAugustine, Ph.D.

OTHER INVESTIGATOR: D. Eckberg, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	0.5
Professional:	0.3
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: One objective of the current program is to study the properties of the rat lung membrane-bound enzyme. This enzyme cleaves the nicotinamide-ribose link of NAD. The enzyme has been shown by others to have transglycosidase activity (the reverse reaction) *in vitro* and incorporate drugs such as isoniazid (INH) into the parent NAD molecule in exchange for nicotinamide. A second objective is to examine whether or not transglycosidase activity observed *in vitro* is a real process *in vivo* as judged by the formation of an isolatable ^{14}C-INH analog of NAD.

METHODS EMPLOYED: UV spectroscopy, liquid scintillation and paper chromatography were employed.

MAJOR FINDINGS: β-NAD glycohydrolase appears to occur exclusively in membrane fractions of the rat lung. The membrane-bound enzyme had a broad pH optimum (5.9-6.9). Nicotinamide was a potent inhibitor *in vitro*; whereas isoniazid was a poor inhibitor. This, then, makes the enzyme similar to the human lung enzyme which is also isoniazid "insensitive." The enzyme had negligible activity in degrading NADP and was essentially unable to degrade NADH, NADPH or α-NAD. Enzyme activity also seems restricted to lung "parenchyma." "Free" cells, or lung alveolar macrophages, isolated from the rabbit, had no detectable enzyme activity, either as intact cells or as sonicated whole cell suspensions. *In vitro* studies using the 25,000 g membrane fraction, NAD and ^{14}C-INH revealed

the formation of ^{14}C-INH-ARPPR. This labeled analog could also be isolated by acetone precipitation of the trichloroacetic acid extract of the homogenized lung of rats injected with ^{14}C-INH. Isotope-activity of the extract spotted and developed on paper chromatograms gave a peak having an identical Rf to that of the material formed in vitro. No reduced dinucleotide could be detected in the extract by UV absorbance. The decay rate of total ^{14}C activity in the rat as determined by tissue oxidations was approximately 25-30 minutes, whereas the decay rate of the INH-IRPPR analog was 60-70 minutes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An investigation of lung tissue levels and extent of retention of the isoniazid analog of NAD by the lung could bring insight as to whether or not this process is important in the mechanism of action or toxic responses observed for this drug or related chemicals.

In addition, the fact that the alveolar macrophage was devoid of this enzyme suggests that these cells do not originate from the lung epithelium and probably do not extensively utilize anaerobic glycolysis. Such information could give us a basic understanding about these macrophages, which are vital to pulmonary defense and clearance.

PROPOSED COURSE: A manuscript giving this information in detail is currently being prepared for publication and no further aspects of this enzyme will be investigated in the near future. However, the coupling of isoniazid to proteins in vitro and in vivo by the action of hepatic and lung transglutaminases is currently being investigated as part of a series of studies of mechanisms of how chemicals, both drugs and environmental chemicals, become covalently bound to tissue small-molecular weight compounds, macromolecules and membrane components.

PUBLICATIONS

DiAugustine, R. P., Gipson, S., and Howell, D.: Lung NAD glycohydrolase. Formation of the INH analog of NAD in vitro and in vivo. Federation Proc. (1973).

Serial No.: NIEHS-P-025
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Hepatic Transglutaminase"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATORS: R. P. DiAugustine, Ph.D., and T. Abe, Ph.D.

OTHER INVESTIGATOR: D. H. Eckberg, B.S.

COOPERATING UNITS: None

MAN YEARS:

Total:	1.4
Professional:	1.2
Other:	0.2

PROJECT DESCRIPTION

OBJECTIVES: Transglutaminase catalyzes the coupling of selected amines to the side chain γ-carbonyl group of glutamine with the liberation of ammonia. A typical reaction, for instance, would be the coupling of putrescine to a protein containing "exposed" glutamine residues, such as casein. Calcium is usually required for enzyme activity. This enzyme activity is present as Factor XIII in plasma and has been shown by other workers to catalyze the $\varepsilon(\gamma$-glutamyl) lysine bonds between fibrin monomers. Factor XIII, prior to the sequential clotting reactions, exists as a zymogen or pro-enzyme in plasma. However, this is not the case for the hepatic enzyme which is of a different molecular weight and composition and requires no proteolytic action on the enzyme to evoke activity. This "hepatic" enzyme is apparently quite ubiquitous and various forms have been found in the lung, spleen, muscle and skin of mammals. The $\varepsilon(\gamma$-glutamyl) lysine bond obviously confers much structural constraint or rigidity on participating protein monomers. The resulting cross-linked protein should be much less soluble than the reactants, accounting for a role for this enzyme in fibrin formation and hair or wool cross-linking. The objectives of the present program are to:

(1) purify and characterize rabbit liver transglutaminase

(2) determine what proteins in the hepatocyte act as acceptors for amines such as putrescine or isoniazid in the presence of Ca^{++} and transglutaminase

(3) to search for the presence of the ε(γ-glutamyl) lysine bonds in the hepatocyte and prove that such bonds are formed by the liver enzyme. If such bonds are detected, an attempt shall be made to isolate the complete proteins containing such bonds

METHODS EMPLOYED: UV spectroscopy, pH stat, column chromatography, liquid scintillation, fluorescence spectroscopy, electrophoresis, ultracentrifugation and amino acid analysis were employed.

MAJOR FINDINGS: Purification of rabbit liver transglutaminase was made by the following procedure: Rabbit liver homogenate was centrifuged at 110,000 kg for 60 minutes. The supernatant fraction was treated with enough acetic acid to give a pH of 5. The precipitate which formed was extracted with pH 6.5 acetate buffer solution and then a fraction precipitated between 30-50% sat. $(NH_4)_2SO_4$(pH 7) was made from the dialyzed extract and was further purified by DEAE cellulose, Sephadex G-200 and DEAE-Sephadex column chromatography. Purity was indicated by a single homogeneous bond on gel electrophoresis. The apparent molecular weight of the enzyme was 80,000 as determined by gel filtration. CBZ-glutaminyl-glycine (synthetic acceptor) and hydroxylamine (donor amine) were used as an assay, but the enzyme was judged extremely unstable. However, purification did not appear to make the enzyme more unstable. Ca^{++} was required for activity and the pH optimum was 6.5.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. The fact that a number of amine-containing drugs such as isoniazid and hydralyzine can be coupled to certain proteins by transglutaminase makes this enzyme a suitable candidate for drug- or chemical-induced antigen formation in vivo. Some clinical evidence has already been reported suggesting that antibodies to plasma transglutaminase are responsible for isoniazid-induced hemorrhaging. Knowledge about what types of amines can be potentially coupled to proteins should give some predictability to the study of chemical and drug hypersensitivity.

2. The ε(γ-glutamyl) lysine cross-link is a recently discovered covalent protein-protein interaction that appears to be more widespread than was previously assumed. If this bond can be found in hepatocyte plasma membranes it would give us a new and important component to consider in the study of physical, chemical and functional properties of membranes. For instance, what process controls the catalysis of the cross-link in membranes? Calcium influx to the cell? From where are the proteins derived to make the cross-link? Is this cross-link present only in certain membranes? What property does this protein give to membranes?

PROPOSED COURSE:

1. To further determine the biochemical and physical properties of rabbit liver transglutaminase.

2. To identify soluble and membrane proteins capable of acting as acceptors for transglutaminase-catalyzed reactions.

3. To search for the presence of the $\varepsilon(\gamma$-glutamyl) lysine cross-link in hepatocyte proteins. If this isopeptide is found, a survey will be made for this compound in various cell types and cell conditions, e.g., normal vs. tumor.

Serial No.: NIEHS-P-026
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "The Effects of Chronic Intermittent Inhalation Exposure of Animals to Airborne Pollutants"

PREVIOUS SERIAL NUMBER: None

PRINCIPAL INVESTIGATOR: R. T. Drew, Ph.D.

OTHER INVESTIGATORS: Becton-Dickinson Research Laboratories

COOPERATING UNITS: Environmental Biology and Chemistry Branch and Environmental Biometry Branch

MAN YEARS:

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: To determine chronic inhalation toxicity of airborne pollutants, alone and in combination with other materials by inhalation and other routes of administration.

METHODS EMPLOYED: Rodents will be exposed to the airborne materials and the toxicological effects will be assessed using several of the following criteria: morphological changes in selected tissues, alteration of tissue enzyme or biochemical parameters, cardiovascular and respiratory physiological changes, and alteration in normal kinetics of uptake and excretion of various compounds.

MAJOR FINDINGS: This contract is presently in the final stages of negotiation. The compounds to be used in the first study include SO_2 and aluminum-chloride-hydroxide. The second study may combine ozone and SO_2.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of aerosol products; the current emphasis on highways and automobiles as opposed to railroads; and the recent energy crisis have all added to the already large number of pollutants in the atmosphere. The detrimental consequences of the presence of these pollutants in the atmosphere on the health of the population is difficult to forecast. These animal

studies will provide valuable data which can be used to predict the potential health effects of these air pollutants and possible interaction between them.

PROPOSED COURSE: These studies are just being started.

Serial No.: NIEHS-P-027
1. Pharmacology Branch
2.
3. Research Triangle Park, N.C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Metabolism of Sulfur Dioxide: Characterization and Modification of the Metabolic Pathway"

PREVIOUS SERIAL NUMBER: NIEHS-PT-020

PRINCIPAL INVESTIGATOR: H. J. Cohen, M.D., Ph.D.

OTHER INVESTIGATORS: K. V. Rajagopalan, Ph.D., and J. Johnson, Ph.D., Department of Biochemistry, Duke University, R. T. Drew, Ph.D.

COOPERATING UNITS: Department of Biochemistry, Duke University, Durham, North Carolina

MAN YEARS:

Total:	0.3
Professional:	0.3
Other:	0.0

PROJECT DESCRIPTION

OBJECTIVES: This is an effort to understand and possibly manipulate one mechanism by which animals detoxify a common air pollutant, sulfur dioxide. Studies seek to characterize the enzyme, sulfite oxidase, which metabolizes sulfur dioxide to sulfate, and to determine what factors, both environmental and experimental, serve to control the activities of this enzyme system.

METHODS EMPLOYED: Most studies on the enzyme are performed using visual spectrophotometry to visualize the heme group and using electron paramagnetic resonance (EPR) spectroscopy (in conjunction with Dr. K. V. Rajagopalan) to visualize the molybdenum prosthetic group. Gel electrophoresis has been employed using heme-specific and enzyme activity-specific stains. Nutritional studies employing diets containing tungsten and vanadium have served to elucidate mechanisms of control of the enzyme activity. Immunologic techniques including antibodies against sulfite oxidase have been helpful in correlating functional and structural parameters.

MAJOR FINDINGS:

1. Oral and injected tungsten both reduce the levels of hepatic sulfite oxidase in a dose-dependent way.

2. Sulfite oxidase prepared from rats given tungsten was found to contain 0.35 moles of tungsten per mole of enzyme. This protein, however, had no enzymatic activity. It has the same heme content and molecular weight as that prepared from control rats.

3. Tungsten-treated rats are more susceptible to sulfite toxicity - both after inhalation of SO_2 and after intraperitoneal injection of bisulfite.

4. Xanthine oxidase made from livers of tungsten-treated rats contained neither molybdenum nor tungsten. The enzyme did contain the normal amounts of nonheme iron and flavin and displayed no xanthine oxidase activity but did contain molybdenum-independent DPNH oxidase activity. Antibodies were made against both sulfite oxidase and xanthine oxidase and were found to cross react identically with enzymes prepared from livers of tungsten-treated animals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the mechanisms by which the body detoxifies sulfur dioxide and the factors which control these metabolisms may explain why certain individuals or groups are more sensitive to the deleterious effects of SO_2 and how we can alter the sensitivity to this noxious agent.

PROPOSED COURSE: These studies will be continued in collaboration with Drs. Rajagopalan and Johnson at Duke. Characterization of the enzyme in tungsten-treated animals will be extended. Studies on development of sulfite oxidase activity in newborn animals will be initiated. Attempts to further establish a role for sulfite oxidase in SO_2 will be pursued.

PUBLICATIONS

Cohen, H. J., Betcher-Lange, S., Kessler, D. L., and Rajagopalan, K. V.: Hepatic sulfite oxidase: Congruency in mitochondria of prosthetic groups and activity. J. Biol. Chem. 247: 7759 (1972).

Cohen, H. J.: The use of diaminobenzidine for spectrophotometric and acrylamide gel detection of sulfite oxidase and its applicability to hydrogen peroxide generating enzymes. Anal. Biochem., Vol. 53, p. 208, 1973.

Cohen, H., Drew, R. T., Johnson, J., and Rajagopalan, K. V.: The relationship between sulfite oxidase activity and the acute toxicity of sulfur dioxide and sodium bisulfite. Proc. Natl. Acad. Sci. 70: 3655-3659, 1973.

Johnson, J. L., Cohen, H. J., and Rajagopalan, K. V.: Studies of vanadium toxicity in the rat. Lack of correlation with molybdenum utilization. Biochem. Biophys. Res. Com., Vol. 56, p. 940, 1974.

Johnson, J. L., Rajagopalan, K. V., and Cohen, H. J.: Molecular basis of the biological function of molybdenum effect of tungsten on xanthine oxidase and sulfite oxidase in the rat. J. Biol. Chem., Vol. 249, pp. 859-866, 1974.

Kessler, D. L., Johnson, J. L., Cohen, H. J., and Rajagopalan, K. V.: Visualization of hepatic sulfite oxidase in crude tissue preparations by electron paramagnetic resonance spectroscopy. Biochem. Biophys. Acta 334: 86-96, 1974.

Johnson, J. L., Cohen, H. J., and Rajagopalan, K. V.: Molecular basis of the biological function of molybdenum. Molybdenum-free sulfite oxidase from livers of tungsten-treated rats. J. Biol. Chem., in press, 1974.

Johnson, J. L., Waud, W. R., Cohen, H. J., and Rajagopalan, K. V.: Molecular basis of the biological function of molybdenum. Molybdenum-free xanthine oxidase from livers of tungsten-treated rats. J. Biol. Chem., in press, 1974.

RESEARCH SERVICES BRANCH

RESEARCH SERVICES BRANCH
Summary Statement

The Research Services Branch provides support services to the Institute in the form of planning and acquiring appropriate facilities; operating, altering and maintaining the facilities; providing sterile glassware and media for the laboratories; providing a safety and security program; designing, fabricating and maintaining research associated instrumentation.

Two contracts for A/E design services have been let for stage 1A of the NIEHS permanent facilities. The first contract is with the joint venture of Max O. Urbahn Associates, Inc./LBC&W, Inc. for the design of approximately 310,000 gross square feet of program space. The majority of this space will be laboratories but there will also be administrative and support space provided. The second contract is with the joint venture of Odell Associates, Inc./Smith, Hinchman and Grylls Associates, Inc. for the design of site development and 96,000 gross square feet of support space. This space will include the power plant, shops, warehouse and other miscellaneous functions. Design is progressing on both contracts and the projected completion date is October, 1975.

A 9600 gross square foot office building is essentially completed at the North end of the NIEHS interim site. This building will house the Library, the Biometry Branch and a 1200 square foot seminar room.

The instrumentation development shop completed over 200 work orders during the past 12 months ranging from very simple tasks to design and fabrication of complex instrumentation. Examples include: a 20 sample filtering device for the immobilization of DNA on nitrocellulose filters either simultaneously or individually; an automated multi-sample innoculation device for Mutagenesis; and several lung perfusion devices for Pharmacology.

Laboratory requirements for glassware and media have been relatively stable this year. Mutagenesis continued to be the major user of media. Previous contamination problems with PPLO in tissue culture systems has not appeared this year.

OFFICE OF THE ASSOCIATE DIRECTOR FOR
SCIENTIFIC INFORMATION AND COMMUNICATION

OFFICE OF THE ASSOCIATE DIRECTOR FOR
SCIENTIFIC INFORMATION AND COMMUNICATION
Summary Statement

Scientific Liaison with Categorical Environmental Programs

The survey of Federal programs dealing with environmental health research made for the OST-CEQ Ad Hoc Committee in FY 1972 established a network of personal communication that has stood the Institute in good stead this year. In some instances, copies of project sheets and other program documents are now sent automatically by activities that hardly communicated at all before. In most other cases a telephone call is all that is necessary to get information. Periodic visits serve to keep our files reasonable current. Time and the activities of a previous staff member have helped to break down an earlier suspicion that NIEHS had predatory intentions. NIEHS is consulted more frequently by other activities on matters of mutual interest. Lack of spontaneity is noticeable with only one major program. Direct scientist-to-scientist communication has been greatly increased.

Quick Retrieval System for Scientific Information

The principal target for this activity is now the project information received from other programs. A fairly quick mode of access, although still manual, has been developed. The system can be readily automated if the conduct of a survey is authorized by higher echelons. A Project Board is maintained giving reference numbers to documents dealing with elements of other programs.

Intramural Scientific Information Service [Including Library]

This continues to expand. A move to new quarters has been accomplished resulting in more efficient operations. A MEDLINE terminal has been installed and put into operation. At present, MEDLINE requests average 25-30 per month.

Scientific Information Evaluation and Reporting Service

This activity is now taking form as a multi-level mode of promulgating considered opinion and authoritative review of complex environmental problems on which only isolated and partial statements were previously available. The four books complied for non-specialists in conjunction with the Fogarty International Center seem to be reaching a fairly wide audience. A very favorable response has been received from the readership of ENVIRONMENTAL HEALTH PERSPECTIVES after six issues. Four more are currently in the working stage or in preparation. Review articles sent to the periodical ENVIRONMENTAL HEALTH PERSPECTIVES are in good demand. NIEHS seems to be developing a reputation for being the source of well-balanced statements on several tricky problems. Participation in conferences

organized by the New York Academy of Sciences has served to brighten this image. Critical comments on draft action documents sent for review by such programs as EPA and NIOSH have earned respect, even when they have not evoked much happiness. Although the intent is peripheral to its mission, NIEHS is now fairly frequesntly asked to participate, and to review the evidence, at sessions in which industry, labor, medicine and science jointly consider some of the more difficult environmental problems.

International Programs

The responsibility of this Office is limited to operation of the bilateral agreement with France relative to pulmonary effects of environmental contaminants. We participated, with the EPA, in the AAAS conference in Mexico on "Science and Man in the Americas" on June 22-30, 1973, and July 1-5, 1973. The June meeting was concerned with "Ecology and Deterioration"; and the July meeting with "Environmental Health in the Americas".

Editorial Committee

The number of papers submitted for clearance over the period July 1 to April 1 was 133. From April 1, 1973 to April 1, 1974, 191 papers were submitted. To date, 550 manuscripts have been cleared.

Office Of the Associate Director for Scientific Information and Communication: Some reorganization of the Office of the Associate Director for Scientific Information and Communication is expected as the Associate Director has retired.

Serial No.: NIEHS-OSI-001
1. Office of Associate Director for Scientific Information & Communication
2. Liaison
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Scientific Liaison with Categorical Environmental Health Programs"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Douglas, H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: Categorical Environmental Health Programs

MAN YEARS

Total:	0.7
Professional:	0.2
Other:	0.5

PROJECT DESCRIPTION

OBJECTIVES: The primary objective is to maintain awareness of relevant scientific and technical activities between the intramural operations of NIEHS and other Federal environmental programs. Formal processes support, but do not satisfy ultimate objectives of liaison -- meeting together, thinking together, working together.

BACKGROUND: One of the reasons advanced for creating NIEHS was the necessity for a facility which would provide the more basic, longer-term, multiple factor research necessary to the development of criteria, standards, and control measures by the categorical environmental health programs. This necessitates continuing exchange of information on problems, needed research, investigative activities, and emergent scientific information between the research and categorical activities. The need was expressed in announcements by the Surgeon General on the implementation of NIEHS, and has been the subject of frequent inquiry by OMB and DHEW representatives.

MAJOR ACCOMPLISHMENTS: The ready flow of project information from agencies across the Federal Government concerned with environmental health has continued. The currency of files, including relevant legislation, is checked at periodic visits to the agencies. Pending clarification of a continued survey under the auspices of higher echelons, the information has not been coded or indexed in detail. Retrieval is possible only by manual search.

PROPOSED COURSE: Continued acquisition of project material from relevant programs combined with updating at personal visits.

Serial No.: NIEHS-OSI-002
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Quick Retrieval System for Scientific Information"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Richard K. West

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.4
Professional:	0.7
Other:	0.7

PROJECT DESCRIPTION

OBJECTIVES: The objective is the development and operation of a system for quick retrieval of information on scientific topics relevant to NIEHS programs.

BACKGROUND: The relatively unstructured nature of information obtained from categorical programs (see Project OSI-001) and other sources imposes the need for some system of retrieving information on a particular subject with speed and precision. The preparation of position and other staff papers (see Project OSI-005) also demands quick assess to important relevant information. Intramural scientists need assistance in securing references, abstracts, and original documents relating to topics of immediate interest. It is desirable that one system be established for handling information acquired to meet these internal needs.

MAJOR ACCOMPLISHMENTS: The system has been modified to concentrate on project information received from other programs and to be capable of responding to any directive from higher echelons for repeated review of Federal programs.

PROPOSED COURSE: Work will continue on the development of a system for automatic search and compilation of project sheets as directed by higher echelons even though the principal administrator has retired.

Serial No.: NIEHS-OSI-003
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "NEHSC Library"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Ralph Hester

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.8
Professional:	0.3
Other:	1.5

PROJECT DESCRIPTION

OBJECTIVES: To provide necessary library holdings and services for the use of NIEHS personnel.

BACKGROUND: Although the three neighboring universities provide excellent backup library resources, and the services of both the NIH library and the National Library of Medicine are available to NIEHS, a moderate library facility is essential in the immediate vicinity to minimize the time spent by scientists in travel and absence from active work, and to facilitate consultation of relevant material.

MAJOR ACCOMPLISHMENTS: We have recently moved the library facilities to Building 18 which should better facilitate the use of the library due to a more organized system. There are now 211 periodicals regularly received. Demands for interlibrary loans average 445/month. The annual purchase budget is $44,000.

PROPOSED COURSE: Increased coverage as new Branches are established in the Institute.

Serial No.: NIEHS-OSI-004
1. Office of the Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Intramural Scientific Information Service"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Ralph Hester

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.7
Professional:	0.7
Other:	1.0

PROJECT DESCRIPTION

OBJECTIVES: The objective is to provide relevant scientific information by both routine and demand services to NIEHS personnel.

BACKGROUND: Both the preparation of position and other staff papers, and the consideration of relevant scientific literature by working intramural scientists, requires the acquisition, assembly, and referencing of documentary information from a wide range of sources. Both the Scientific Directorate and the intramural scientists require assistance in (a) using the Quick Retrieval System (Project NIEHS-OSI-002); and (b) going beyond the limited internal library resources (Project NIEHS-OSI-003) to external sources.

MAJOR ACCOMPLISHMENTS: Information Services now constitutes a Section in the functional organization of OSIC, charged with securing such scientific information as is required by Institute members for the various purposes. This includes normal library operations (NIEHS-OSI-003), personal services according to profiled requirements, and meeting demands for special purposes. It provides an essential input to the growing demand for position papers on a variety of subjects (NIEHS-OSI-005). Much of the information secured becomes potential imput to the QRS (NIEHSOOSI-002). A MEDLINE terminal has been installed. It shares a line with UNC and Duke University Medical School libraries. There is an average of 25-30 requests per month.

PROPOSED COURSE: Increased services with access to additional external sources.

Serial No.: NIEHS-OSI-005
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Scientific Information Evaluation and Reporting Service"

PREVIOUS SERIAL NUMBER: Same

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	1.7
Professional:	0.5
Other:	1.2

PROJECT DESCRIPTION

OBJECTIVES: The objective is to evaluate the relative significance of various environmental stresses and to prepare reports on selected topics in environmental health.

BACKGROUND: a. Efficient allocation of Institute resources to specific investigations presupposes a knowledge of the range of environmental situations that affect man, the relative seriousness of their effects, the feasibility of ameliorating the results, and the extent of investigative attention already being given to the significant situations. b. The Directorate is frequently called upon to present position papers on various environmental health matters of national import.

MAJOR ACCOMPLISHMENTS: Six numbers of ENVIRONMENTAL HEALTH PERSPECTIVES have been issued. Material for four more numbers has been assembled. Authorization has been given to continue this as a permanent publication. A Handbook on Environmental Physiology for the American Physiological Society is almost ready for press.

PROPOSED COURSE: Continue with ENVIRONMENTAL HEALTH PERSPECTIVES, and organization of the Handbook on Environmental Physiology. Work with the University of Florida Medical School to produce publications on environmental medicine.

Serial No.: NIEHS-OSI-006
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Participation in International Programs"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-009

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

OBJECTIVES: The objective is to participate in those aspects of international programs operating through DHEW that are relevant to the NIEHS mission.

BACKGROUND: With the transfer of the categorical environmental programs out of DHEW, NIEHS becomes a mainstay of DHEW interests in environmental health. This applies particularly to international programs, in which environmental aspects are getting increasing attention. Close cooperation is maintained in this connection with the Fogarty International Center and with the Office of International Health.

MAJOR ACCOMPLISHMENTS: Under the bilateral agreement with France, Dr. Pham came to the USA for the period April 30 to June 15, 1973. Dr. B. Ferris (Harvard) visited France from September 6 to October 30, 1973. Dr. Voisin came from France and stayed from February 5 to March 9, 1974. Dr. LeRoy Lapp (NIOSH) is to visit France during FY 1974.

Dr. Douglas H. K. Lee participated in an AAAS Conference in Mexico City from June to July, 1973.

A Soviet delegation visited NIEHS for a week (March 25-29, 1974) under the bilateral agreement with DHEW. Several other Soviet delegations visited at various times. In addition, several other foreign visitors have been received by NIEHS.

PROPOSED COURSE: Continue the bilateral arrangements with France for the present. Cooperate with other developments as necessary.

Serial No.: NIEHS-OSI-006
1. Office of Associate Director for Scientific Information & Communication
2.
3. Research Triangle Park, N. C.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

PROJECT TITLE: "Participation in International Programs"

PREVIOUS SERIAL NUMBER: NIEHS-OSI-009

PRINCIPAL ADMINISTRATOR: Douglas H. K. Lee, M.D., Ph.D.

OTHER ADMINISTRATORS: None

COOPERATING UNITS: None

MAN YEARS

Total:	0.5
Professional:	0.2
Other:	0.3

PROJECT DESCRIPTION

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OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

SUMMARY STATEMENT

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

RESEARCH GRANTS

The number of research grant applications assigned to the NIEHS for review in FY 1974 increased by 9 percent over FY 1973, and the number recommended for approval increased by 16 percent. The $12.9 million available for this activity supported 91 noncompeting continuations and 98 new and competing continuation projects. The 98 competing awards represented a 158 percent increase over the 38 supported in FY 1973, and included 49 projects awarded from funds restored to the NIEHS appropriation from the release of impounded FY 1973 funds. In addition, 7 Research Career Development Awards were made.

TRAINING PROGRAMS

The $3,397 million in FY 1974, including a restoration of impounded FY 1973 funds, allowed the Institute to support 28 training programs at approximately the levels supported prior to the announced phaseout of NIH training grants. Training grant awards included funds for the restoration of programs to previously committed levels for the July 1, 1973 to June 30, 1974 training period, and for the continuation of training activities for the period beginning July 1, 1974.

RESEARCH MANPOWER DEVELOPMENT

On July 9, 1973, Secretary Weinberger announced Administration plans to establish a new health manpower development plan, which supercedes the previous training program scheduled for phaseout as announced in the April Administrative Report. The program is for research training only, with primary emphasis on support of individuals rather than of institutions. Support is limited to postdoctoral candidates for a total of three years. Stipends will be $10,000 for the first year, with $600 increases during the second and third years. Upon request, institutions may be provided training expenses of up to $3,000 a year for each fellow. The award will contain the condition that recipients who do not engage in fulltime research and/or teaching careers of acceptable character subsequent to their training for a period equal to their training support will be required to reimburse the Federal Treasury. Program policy and central management will come from the Office of the Director, NIH.

The objective of the NIEHS fellowship program is to increase the number of career scientists with expertise in the assessment of environmental factors in health, assessment of risk-benefit ramifications of such factors in terms of socio-economic considerations, and in development of preventative and therapeutic health care programs consistent with competing human needs. The

plans call for support of 5 ongoing fellowships and for initiation of 40 new fellowships at a total cost of $606,000 during FY 1974. Fifty and 60 additional awards will be made in FY 1975 and 1976, respectively, bringing the total to 150. Subsequently this number will be maintained by acceptance of new fellows each year to replace those leaving the program.

Specific areas covered by the program are (1) environmental toxicology, (2) environmental pathology-pathophysiology, (3) environmental epidemiology and statistics, and (4) environmental biology. Emphasis in environmental biology will be on the development of methods of testing and evaluating the mutagenicity of environmental factors in man and lower mammal and their progeny and in human populations. Prior to formal application, applicants must arrange for admission to an appropriate institution and for acceptance by sponsors who will supervise their activities. Appropriate institutions are private or public nonprofit organizations and government agencies that have the staff and facilities to provide the proposed research training.

Applications are reviewed initially by committees composed of consultants from scientific fields, primarily from the academic community. The evaluation is based upon the qualifications of the applicant and his potential for research.

UNIVERSITY-BASED CENTERS

University-based Centers are an integral part of the strategy of NIEHS in serving optimally the national needs in environmental health research. They evolve as a consequence of demonstrated ability of a group of scientists within the same institution to perform multifaceted, high priority research and to develop a substantial portion of the health manpower to meet the future needs of the nation. During FY 1974, the National Institute of Environmental Health Sciences supported six Centers with funds totaling $4.0 million. Plans have been made to establish an additional Center during the coming year with outstanding research capabilities in heavy metal toxicology and related high priority areas.

Communication between the Centers and the Institute was maintained at an effective level through biannual meetings of the Center Directors, frequent visits of telephone contacts by Extramural staff and through the NAEHS Council's Subcommittee for University-based Centers. The Subcommittee provides liaison between the Institute, the Advisory Council, and the Center Directors. It visits each Center in turn to discuss research problems and accomplishments, new environmental concerns, and program directions.

Dr. Earl P. Benditt, Chairman of the Department of Pathology, University of Washington at Seattle, replaced Dr. Edward M. Cohart of Yale University as chairman of the Subcommittee upon expiration of Dr. Cohart's Council appointment at the end of September. Dr. Richard L. Riley, Professor of Environmental Medicine, School of Public Health, Johns Hopkins University, was appointed to the Subcommittee to fill the vacancy. In addition to regular sessions held jointly at the Center Directors' Meeting, the Subcommittee was convened separately three times since the last reprot. On March 5-7, 1973, it attended the Special Study Session deliberations on the

Center grant proposal submitted by Dr. Irving Selikoff at the Mount Sinai School of Medicine. The Center grant was approved and is currently funded by the Institute. A second meeting was held September 9-11, 1973, in Nashville, Tennessee to review the Center in Environmental Toxicology, Vanderbilt University and to deal with the request of the University administration to name Dr. Robert A. Neal permanent Director of the Center to replace Dr. Frank Blood, deceased. The Subcommittee concurred with the request. The concensus opinion was that, with correction of two weaknesses identified in the program, the Center would develop into a valuable environmental health resource under Dr. Neal's leadership. The Subcommittee also visited the Center for Environmental Medicine of New York University at Sterling Forest, New York February 10-12, 1974, in connection with the application for renewal of the grant. Despite some reservations about minor parts of the program voiced by the Special Study Section, the Subcommittee continues to view the Center as an outstanding environmental health resource.

Biannual meetings of Center Directors were convened at Oregon State University, Corvallis, Oregon on May 21-22, 1973, and at Research Triangle Park, North Carolina on October 15-16, 1973. The purpose of the meetings is to provide a forum for exchange of information and discussion of mutual interests and concerns among the Directors, the NAESC Center Subcommittee, the NIEHS staff, and invited staff of other government agencies with responsibilities in environmental health and related areas. The agenda for the two day meeting in May included summary reports on health research of the EPA National Environmental Research Center (NERC) and the USDA Forest Service in Corvallis, a symposium on recent advances in pharmacokinetic modeling, a description of the NIEHS science program, and a review of federal administrative and scientific developments and meetings related to environmental health. The agenda for the October meeting included reviews of Center program highlights by each of the Center Directors and of NIEHS Intramural research by Intramural branch chiefs. These reviews are part of a continuing effort to improve liaison and information exchange among Institute staff, Center Directors and Institute advisory bodies. Plans have been made to further broaden this exchange by encouraging attendance and participation of senior staff members at each future Center Directors meeting.

SUPPORTED RESEARCH HIGHLIGHTS

During the year, grantees made substantive contributions to each of the categorical programs of the Institute. The etiological relations of bioactive environmental agents, some new and some with long-standing usage, in human health were illuminated further. Progress was made in understanding the basic toxicological and pathological events in pulmonary and cardiovascular diseases, reproductive and genetic anomalies, neurological disorders and various other syndromes ascribable to environmental factors. Parallel advances were made in analytical methodology for specific toxicants and noxious dusts and in development of quantitative test systems for carcinogenesis and mutagenesis. Highlights from a number of program which illustrate these achievements are presented herewith.

RESEARCH HIGHLIGHTS

ETIOLOGY

The number and variety of toxic substances released into the environment each year increases by an estimated 1000 compounds. Because of the nature of modern society, it is dependent upon technology for jobs; transportation; control of diseases; production of food, fiber and energy; and the preservation of foods and material. Thus no segment of the population can escape some contact with potentially dangerous substances. It is imperative, therefore, that dangerous agents present in the environment, or proposed for future release, be identified and that the consequences of human exposure in terms of overt and covert diseases and physiological disorders be understood. Such knowledge is the first defense against further degeneration of environmental quality with its consequent toll on the quality of human health. Development of such knowledge and understanding is the purpose of this program.

Air Pollutants

A large portion of environmental agents have a primary interface with the lungs and interact directly at or near the portal of entry. Interrelationships between many of these agents and important diseases such as lung cancer, emphysema, chronic bronchitis, pneumoconiosis, asbestosis, farmer's lung and byssinosis are already clearly established. During the past year substantial new information about the etiological role of specific agents in these and other disorders was developed.

Ozone. Significant findings regarding oxidant air pollutants were reported from several NIEHS grantees. Dr. Bernard Goldstein of the New York University Medical Center found that carbonyl compounds are increased in the blood of experimental animals exposed to ozone. Such compounds are numerous and complex and are presumed to be important as secondary, if not primary, factors in the progression of ozone-induced lung damage. Efforts are being made to isolate and identify the compounds so that each of them can be evaluated individually as factors in the etiology of ozone damage.

He found also that various endogenous substances and even genetic makeup of the organism can influence ozone toxicity. p-Aminobenzoic acid, chlorpromazine, phenobarbital, and allylisopropylacetamide modify toxicity. Phenobarbital increases toxicity, apparently through induction of cytochrome P-450, which, in turn, facilitates formation of peroxides from certain lipids. Allylisopropylacetamide, on the other hand, reduces the level of cytochrome P-450 in cells and concomitantly protects against ozone toxicity.

Substantial differences were found in ozone susceptibility among inbred strains of mice. In at least one case studied, resistance appears to be

inherited as a dominant characteristic. Whether this is related principally to differences in cytochrome P-450 or to lipid characteristics is not yet known.

NO_2. Studies of related phenomena involving NO_2 and vitamins A and E were carried out by Dr. Saari Csallany at the University of Illinois. She found that NO_2 inhalation accelerates lipid peroxidation and formation of age-related pigments called lipofuscins. Moderate doses of vitamins A and E reduced peroxidation and lipofuscin formation. This suggests that high levels of certain vitamins and perhaps other natural antioxidants may be useful in preventing the peroxidative damage induced by atmospheric oxidants.

SO_2. In studies of another prevalent gaseous pollutant, SO_2, Dr. Edward Palmes has provided further data relating to the mechanism of SO_2 transport to other parts of the body. Dr. Palmes previously reported the detection of thiolsulfonates in the blood of animals inhaling SO_2. He has now shown that sulfite reacts with disulfides in mammalian plasma, producing stable S-sulfonates. The reaction goes essentially to completion,and plasma S-sulfonate concentration in SO_2-exposed rabbits increases upon continuous exposure at 10 ppm in air until an equilibrium with uptake is reached, usually within 3 to 5 days. At that time the S-sulfonate level was found to be substantially above endogenous levels. Upon withdrawal from exposure the S-sulfonate levels declined, with a mean half-time of about 3 days. The rapid clearing is believed to reflect the diffusion of small sulfonates, S-sulfo-cysteine in particular, from the blood vessels. The slower phase of clearance, which accounts for some 10 to 20% of the total, apparently represents nondiffusible protein S-sulfonates.

The significant aspect of the conversion of sulfite into reasonably persistent protein S-sulfonates is the potential for the transport of inhaled sulfur dioxide or ingested sulfite to other areas of the body. Since the protein S-sulfonates are nondiffusible, they remain in the blood stream until dissociated. Dissociation occurs gradually upon physical contact with cellular or plasma thiols. At that time the sulfite may be released and eliminated or undergo subsequent reactions with essential thiol proteins. It is suspected that direct S-sulfonate-thiol protein exchange may occur also, since analogous reactions *in vitro* are well established. The expected consequence of such reactions is inactivation of enzymes or coenzymes with functional sulfhydryl groups.

Collaborative studies on 12 human subjects continuously exposed to SO_2 were carried out at Ohio State University's Department of Preventative Medicine to determine if SO_2 reacts in a similar manner in humans. The experiments show that the levels of S-sulfonate increase in parallel to the concentrations of SO_2 to which the indivuduals are exposed. The blood level increase was about 1.5 nmoles for each ppm increase in SO_2 concentration in the air. Studies are now in progress to determine the nature of the proteins involved in the thiolsulfonate reaction and the consequences of inactivation of the proteins in terms of chronic physiological disorders.

Benzene. Dr. Robert Snyder of Thomas Jefferson University has made further progress toward localizing and understanding the toxic action of the important industrial solvent benzene. He has developed a method for detecting very early signs of benzene toxicity. A single dose of benzene in the mouse depresses incorporation of ^{59}Fe into an early precursor of the erythrocyte in the bone marrow. There is a lag time of some 12 hours after benzene exposure before the depression of erythrocyte production becomes evident. Through appropriate correlates in erythrocyte formation, precise identification of the cell type involved and the elucidation of the biochemistry of suppression should be possible. The depressant effect disappears after about 72 hours, which indicates that the bone marrow is not permanently damaged. Further studies are expected to provide a basis for preventing or antagonizing the effect.

Metabolic studies in mice suggest that only one major metabolite, e.g., phenol, is formed from benzene in the body and that the compound is rapidly excreted in the urine as the sulfate or glucuronide conjugate. Metabolism is stimulated by pretreatment with microsomal enzyme inducers, such as phenobarbitol, or by benzene per se, which apparently acts as a self-inducer. Efforts are now being made to find antagonists and enzyme inducers that will afford protection of erythrocyte biogenesis against benzene and similar toxicants.

Chlorinated Bisphenols. Chlorinated bisphenols, especially hexachlorophene, are highly toxic to mammals and act as powerful hemolytic agents. They are found in virtually every home in soaps and cosmetics. Although the compounds are considered to be safe from the standpoint of percutaneous toxicity, poisoning from accidental ingestion is a danger to children. Moreover, convulsions and temporary neurological disorders have been reported in burn patients treated topically with hexachlorophene. To provide further basic knowledge as a guide in assessing potential dangers of this class of compounds, Dr. D. R. Buhler of Oregon State University has conducted a number of studies on distribution patterns, metabolism and mode of action of hexachlorophene in adult, weanling and newborn rats. He found the compound to be highly persistent in the environment and slowly metabolized in the body. It is accumulated in the small intestine, liver, stomach and kidneys of the adult and weanling rat. However, absorption was much slower in the newborn and, of that absorbed, significant amounts accumulated in the lungs. This suggests that metabolic defenses in the lung of the newborn may be poorly developed, increasing the hazards of exposure at that stage.

Hydrazine. Since the use of hydrazines is rather restricted and localized, the potential for exposure is not considered widespread. However, some individuals who work with them are exposed to rather high levels. Thus Dr. F. N. Dost at Oregon State University has investigated the steady state intoxication by hydrazine and certain methyl derivatives. He found that the compounds produced certain metabolic lesions characteristic of diabetes. Sublethal doses of methylhydrazine caused profound deficits in carbohydrate metabolism and changes in the activity of specific glucose metabolizing enzymes in the liver. The changes are reversed almost immediately by administration of pyridoxine HCl or insulin. Because of the apparent mimicking of diabetic changes, these compounds should be considered dangerous to diabetics and others with anomalies in carbohydrate metabolism.

Airborne Fluorides. Other workers at Oregon State University under the direction of Dr. F. W. Adams have been concerned for several years with the hazards of fluorides, which are released in great quantities from aluminum refining plants. Much of the information they have developed concerning mammalian effects is now available.

Among the superficial effects of exposure to hydrogen fluoride is mottling and banding of incisor teeth. Tooth discoloration is also found in humans drinking water with a fluoride content of 1.5 to 2 ppm. More profound effects include abnormal growth on the surface of certain bones (termed exostosis) in experimental animals. This occurs also in man in extreme cases of industrial or endemic fluorosis. Fluorides entering through the respiratory tract or through ingestion produce the same effects and are accumulated in similar fashion in bone.

In reproductive studies, low levels of fluorides (about 1 ppm) in the atmosphere did not visibly affect reproduction or postweaning growth in hamsters, nor did fluorides that traversed the placental membrane in pregnant hamsters have a lasting effect on the young. However, persistent high fluoride levels (5 to 300 ppm) reduced the life span of exposed hamsters by about 25%. Experimental dams under these conditions exhibited increased irritability, and the percentage of weanlings and the weights of the pups at weaning was reduced. The effects on the young may reflect significant behavioral and physiological effects on the mothers manifested as intolerance to the young or restricted milk production. Levels of the order of 1 ppm in air appear to be tolerated.

Dichloroethylene. A number of chlorinated monomers used extensively in industry in the synthesis of polymers have been implicated in liver toxicity and carcinogenesis. Dr. Sheldon Murphy of Harvard University has carried out studies on a number of such agents, including 1,1-dichloroethylene (DCE). This monomer is used in the manufacture of Saran-type plastics and has been reported to be a contaminant of submarine and spacecraft atmospheres, as well as in occupational environments. Workers exposed to as little as five ppm of vinylidene and vinyl chlorides in air are reported to show evidence of hepatic injury.

In addition to studies on the mechanism of hepatotoxicity of these types of compounds, which is generally similar for the group, Dr. Murphy has placed primary emphasis in recent studies on nutritional factors and trace metals that influence toxicity. He has shown that fasted rats are notably more susceptible to lethal and hepatoxic action of the compounds than animals fed ad libitum. The difference was of the order of 2000-fold. Rats fasted overnight had much lower glutathione levels than nonfasted animals. Moreover, treatment of nonfasted animals with diethylmaleate, which reduced liver glutathione by about 50 percent, greatly enhanced the toxicity of DCE. This clearly demonstrates that glutathione acts as a protective agent against these types of hepatotoxins and emphasizes the importance of diet in the well-being of individuals who must come into contact with these kinds of agents as part of their jobs. It also provides basic information about the kinds of compounds that might have therapeutic or prophylactic value.

Carbon Monoxide. The most nearly ubiquitous gaseous toxicant is carbon monoxide. This substance touches the lives of everyone who is dependent upon combustion for home heating, transportation, manufacturing, and other essential activities. However, only under special circumstances does the concentration and exposure cohorts converge to present immediate dangers to human health. Unfortunately, the number of such circumstances are substantial; and, during the present shortage of convential home heating fuels, which may lead to makeshift and poorly constructed alternative or supplemental devices, the dangers could well be compounded. Population groups of greatest concern include cigarette smokers, workers involved in automobile traffic control, mechanics working in closed buildings, firefighters, certain factory workers, and, of course, individuals in substandard homes with improperly vented heating devices. In view of these situations, NIEHS supports a substantial number of epidemiological and basic studies designed to provide a better understanding of the extent and nature of the hazard presented by inhalation of carbon monoxide alone and in combination with other toxic agents.

Dr. Richard Riley and his colleagues at Johns Hopkins University have carried out extensive studies on many aspects of carbon monoxide distribution and intoxication. Although CO produces a number of physiological changes in the body, it is generally agreed that one of the more important effects is the reduction in oxygen-carrying capacity of the red blood cells. This results from preferential binding of CO to the oxygen binding sites of hemoglobin to form the relatively stable complex carboxyhemoglobin.

Epidemiological studies of cigarette smokers show that 35 to 40% of the hemoglobin may be in the form of carboxyhemoglobin during periods of heavy smoking. Fortunately, clearance of CO is relatively rapid so that the time during which so much of the hemoglobin is unavailable for carrying oxygen is generally short. Nevertheless, in heavy smokers, it was found that 3 to 15% of the hemoglobin is continually in the nonfunctional carboxyhemoglobin form.

The consequence of reduced oxygen carrying capacity is variable from individual to individual and is related to other disorders. Patients with circulatory insufficiencies and with various forms of anemia are most susceptible to physiological disorders resulting from oxygen insufficiency, and data are now available which show significantly higher mortality rates in smokers with circulatory and blood disorders.

CO effects other than a simple reduction of oxygen-carrying capacity of the blood are also becoming evident. It has been shown that CO combines with muscle myoglobin, cytochrome oxidase, cytochromes A_3 and P-450, catalase and peroxidases, i.e., many cellular constituents containing the heme moiety. Dr. John T. Fales of Johns Hopkins University has examined the possible effects on muscle myoglobin further, since this compound is critical in arterial and heart function. In work with cat papillary and mouse soleus muscle, he found no evidence that muscle myoglobin contributes significantly to oxygen transport into muscle. However, he showed that CO decreases the isomeric tension (muscle function) by about 30%. Whether the effect was a direct consequence of interaction with myoglobin, cytochromes involved in energy production, or directly on the contractile apparatus has not yet been

determined. Nevertheless, it is apparent from these findings that CO may indeed damage critical muscle systems quite apart from indirect effects resulting from lowered oxygen transport. Such effects would be expected to be more hazardous in individuals with preexisting heart and arterial muscle damage or incapacitation.

Related studies were carried out by Dr. Walter Ehrlich and his colleagues on the effects of low level exposure on the regulation of cardiac output, coronary flow and other reactions in dogs. They found that exposure to CO sufficient to give 10% carboxyhemoglobin does not drastically change circulatory rate at rest or the adaptation of circulatory rate to work. However, it decreases the critical closing pressure of the heart in dogs at rest and increases total arterial resistance to blood flow. This results in an increase in blood pressure and, of course, in work load of the heart.

Further insight into basic aspects of CO action on endotheloid and muscle cells was provided by Dr. Duane Wenzel of the University of Kansas. Using cultured heart cells, he found that CO-treated cells accumulate 2 to 3 times the "normal" amount of globular lipids. As the pH of cultured heart cell medium was lowered, there was a marked increase in globular lipids in the cells. These observations may have some bearing on the accelerated rate of atherosclerosis and other cardiovascular problems, particularly in cigarette smokers.

Dr. Zoltan Annau of Johns Hopkins has been concerned with possible behavioral effects of CO at low levels. His studies thus far indicate no detectable acute effects on rats at air concentrations of CO below 500 ppm. However, long-term studies suggest that moderate CO levels eventually lead to effects on the central nervous system. These are not due simply to the decreased oxygen-carrying capacity of the blood but may involve irreversible changes in cells and tissues.

Synthesis of the information assembled through basic studies, and through epidemiological studies of special groups subject to high CO burdens, leaves little question that CO is an environmental agent of considerable impact upon the human population. Its effects are much more profound than the limitations it imposes upon the oxygen-carrying capacity of the blood. Directly or indirectly, it may contribute to fatty accumulations in the blood and in arterial and heart muscles and, perhaps more importantly in some situations, it may interfere directly in energy metabolism in vital muscles, aggravating preexisting cardiovascular diseases and their consequences. Thus in addition to the long recognized fact the CO is an acute toxin to be reckoned with in special situations, it may be an insidious etiological factor in long-term degenerative diseases of the cardiovascular system and possibly of the nervous system.

Asbestos. It has been known for 20 years that there is an increased risk of lung cancer associated with occupational exposure to asbestos. Dr. Irving Selikoff and his colleagues at Mount Sinai School of Medicine in New York City have shown in previous studies that the incidence of lung cancer among members of the asbestos insulation workers union is 6.8 times that of individuals in occupations not associated with asbestos exposure. These first experiments involved 370 men.

In a second study involving all members of the Insulation Workers Union, Dr. Selikoff gathered information about cigarette smoking in addition to asbestos exposure in order to assess both factors in the etiology of lung cancer. He found that among some 2,000 nonsmokers only 2 died of lung cancer. Among some 9000 smokers in the group, 134 died of lung cancer, an increase of about 14 per 1000 workers. It seems clear, therefore, that workers at risk of lung disease through exposure to asbestos are put at substantially greater risk by additional lung burdens of cigarette smoke and other environmental pollutants.

Since the initial findings by Dr. Selikoff and his colleagues, which establish clearly the occupational hazard of asbestos, consideration has been given to quantitative aspects of the asbestos problem. Through its various uses asbestos is ubiquitous among industrialized societies throughout the world. Thus, the question is no longer who is exposed but to how much is one exposed, how much is dangerous, and how long after initial exposure does overt disease begin to appear.

As a basis for quantitative evaluation of asbestos fibers in the environment and in the body, Dr. William Nicholson and his coworkers at Mount Sinai have developed a sensitive technique for analysis of fibers in tissues, dusts, and other types of samples. The technique involves use of a scanning electron microscope-microprobe analyzer with a resolving capability down to 250 Å. In all tests of the system, its capabilities have exceeded expectations several-fold. Amosite fibers of 500 Å can be identified in 30 seconds. Thus, the instrument provides heretofore unavailable capabilities for accurate quantitative work that will greatly facilitate the advancement of understanding of etiological and pathological aspects of microparticulates to which man is exposed throughout his life. The instrument is currently being used to document the intensity, extent, and persistence of asbestos pollution from various sources, the lung burden of asbestos at various exposure levels, and the relationships between body content and various types of cancer.

In the current program, data are being derived entirely from human experience and are intended to be direct and specific. The several cohorts being examined were selected to form an exposure gradient from high to low and include asbestos factory workers, populations in the neighborhood of asbestos factories, families of factory workers, workers with indirect exposure to asbestos (such as roofers and tile setters), and non-exposed workers, such as caisson and tunnel workers, who serve as a control group.

Although the studies are still in progress, data already available establish that significant risk of cancer is associated with direct asbestos exposure in asbestos factories. In addition to asbestosis, excess deaths were in the categories of lung cancer, pleural and peritoneal mesotheliomas, and cancer of the stomach, colon, rectum, and esophagus. Indications are that the asbestosis risk was very much like that seen among asbestos insulation workers.

As yet the data for the other groups under study are incomplete. It is anticipated that longer intervals will be necessary before the onset of significant overt disease due to the lower exposures. However, success in maintaining contact with the groups and in keeping up-to-date records of mortality and disease experience indicate that the study can be carried to a fruitful conclusion.

The data on the factory workers have been analyzed with regard to dose-disease response. They show that there is indeed an important quantitative relationship. For example, among 256 men employed less than three months, lung cancer deaths were approximately 3 times the expected number. Among 294 employed 3 to 11 months, the number was 4 times normal; and among those employed for a year or more, the number was 10 times normal. These data provide the first information, statistically highly significant on a cohort basis with identical exposure, of the existence of a demonstrable dose-response relationship.

The interaction of asbestos dust and cigarette smoking in relation to lung cancer risk was mentioned before and is now well documented. Dr. Selikoff and his colleagues have evidence that such a relationship also exists between smoking and pulmonary fibrosis. In a study of Canadian and U. S. insulation workers, it was found that only 4 of 73 deaths among 2,000 non-smokers was due to asbestosis. Among smokers, 45 of 596 deaths were due to asbestotic fibrosis or associated bronchitis and emphysema.

All of these studies point to an unmistakable involvement of asbestos and cigarette smoking in the mortality of certain industrial workers. Many questions still remain, of course, about interactions between other types of particulates and gases, each of which has already been shown to produce adverse pulmonary effects in its own right. These and related studies directly on human populations are considered of such importance that NIEHS has created a Center at Mount Sinai under Dr. Selikoff's direction to facilitate further studies.

Epidemiological studies of environmentally-related cancer have continued also at Harvard University under Dr. James Whittenberger and his colleagues. Briefly, they have confirmed previous indications of prenatal X-ray as an etiological factor in childhood cancer. Work on the association between coffee drinking and bladder cancer was extended, and new data suggest that studies now in progress will support the earlier findings of increased bladder cancer risk among coffee drinkers. Retrospective cohort studies of retired leather workers confirm previous indications of increased bladder cancer in this group. These studies, like those of the Mount Sinai group, indicate the complexity of social and industrial factors in all walks of life that may have a direct individual and combined effect on many types of cancer.

Studies by Dr. W. E. Morton of Oregon State University indicate other unidentified environmental factors in various kinds of cancer. He originally began an epidemiologic study to correlate the incidence of skin cancer with the distribution of arsenic in Oregon. Contrary to expectations, he found that the arsenic rich region of Lane County, Oregon, was relatively inconspicuous for cancer risk, but that certain cancers appeared to be related to other geographic factors and life situations. Basic cell carcinoma was predominantly an urban disease, whereas squamous cell carcinoma was more equally distributed in urban and rural areas. All skin cancers were less common in the coastal region where the rainy, foggy weather results in low exposure to solar radiation. These studies again indicate the complex factors in cancer.

Heavy Metals. In addition to numerous studies on lead and mercury, which have been dealt with extensively in previous reports, the Institute supports a number of studies on cadmium and nickel. There is growing evidence of important etiological relationships between these metals and certain diseases in man. Cadmium is used in many industrial operations and has been shown to be a potent immunosuppressant and hypertensive agent. Individuals at risk of toxic exposure include those in electroplating, welding, soldering, nickel plating, and in the manufacture of electrodes for vapor lamps, photoelectric cells, and storage batteries. Moreover, the cadmium content of the air of industrial areas is almost always significantly above that in rural areas, so that urban populations are subject to considerable accumulations of the metal and its compounds.

Dr. Paul H. Weswig of Oregon State University and his coworkers have attempted to develop a clearer picture of body levels of cadmium relative to specific disorders, using the rat as a model. In accord with previous reports, they found that the blood pressure of male rats on a basal diet containing a small amount of Cd (150 ppm) increased gradually from about 115 mmHg to 150 mmHg after 16 weeks exposure. The blood pressure of females did not increase significantly. A genetically hypertensive strain of rats with usual pressure of about 160 mmHg showed a dangerous elevation to over 200 mmHg after 16 weeks on the diet. This suggests that individuals with preexisting hypertension may be put at substantial risk by Cd intake.

In these studies, renin and angiotensin-converting enzyme (ACE) were also determined. On addition of Cd to the basal diet, there was a rapid increase in renin from less than 3 units to about 15 units and then a leveling off to about twice normal. There was no effect on ACE with time. This shows a direct correlation between Cd intake and changes in pressure regulatory systems.

In addition to effects on blood pressure, Dr. Michael Wolcott at the University of Alabama has found that Cd affects the humoral immune system. The effects are detectable well before any overt signs of Cd toxicity. They are manifested as a large and rapid increase in the number of antibody secreting cells in the spleen and a large increase in light immunoglobulin chains in the blood and urine. The light chains apparently are produced in abnormal proportion to heavy chains by the altered spleen cells and presumably do not combine in the normal way with the heavy chains to form immunologically active antibodies. The implications of these events in the breakdown of resistance to infectious diseases are profound and are being investigated further.

Insecticides. Studies in a number of laboratories were continued on the possible role of insecticides in metabolic and other disorders and on their mode of action. Dr. Thomas A. Miller of the University of California has provided new comparative information about the action of carbamates, organophosphates and chlorinated hydrocarbons on the nervous system, using the cockroach and housefly as models. With newly developed techniques for measuring flight motor activity and cardio-regulatory activity, he has found that lindane has a marked effect on the cardio-regulatory nerves. Carbamates had no such effect but reduced flight motor potentials. The organophosphates

Monitor^{R} and leptophos had effects similar to those of carbamates but were much less effective than the carbamates in disrupting the nervous system over a short period of time. Studies of this type point to significant differences in the modes of action of the major classes of neuro-active pesticides. They show, moreover, that inhibition of cholinesterases, assumed to be the basis of toxicity of carbamates and orgnaophosphates, is not the only biological effect of these compounds. Thus full understanding of the ramifications of exposure to these compounds in health requires further careful work.

Dr. Herman Kleerekoper of Texas A & M University, using goldfish and other fishes as experimental models, has been able to demonstrate changes in behavioral and sensory patterns due to exposure to sublethal quantities of chlorinated hydrocarbons and organic phosphates. As little as 10 part per billion of DDT in ambient waters caused noticeable changes in exploratory patterns and reduced activity of goldfish placed in a new environment. Whether similar changes in behavioral patterns of man occur upon exposure to the compounds has not been determined. However, since the nerve functions in man and fish are similar, studies of this type indicate the potential of the compounds, independent of modifying factors and dose considerations, to influence man's behavioral and learning patterns.

Natural Toxins

Natural toxins with which man may come into contact come from a wide variety of living organisms, including higher plants, lower animals, fungi, algae and bacteria. Man has learned through experience to avoid many of these. There are others, however, which are not easily avoided or detected because they may occur intermittently in food and water or become problems through accidental contact with the poisonous organism per se.

Zootoxins. In the latter category are bites and stings of snakes, insects, arthropods, and various marine animals. Dr. Richard Blanquet of Georgetown University has made considerable progress toward characterization of the nematocyst toxins of two species of medusa (jelly fish) and toward understanding the action of the toxins in vertebrate neuromuscular, respiratory and cardiovascular systems. The two species are both potent stingers and one, Chrysora sp., is extremely abundant in the Chesapeake Bay and along its beaches during the summer months. Due to the severity of the stings, public beaches are sometimes forced to close. Treatment is often ineffectual due, in part, to a lack of knowledge of the chemical nature of the toxin or its mode of action. Human encounters with other species are at best unpleasant and on occasion have resulted in death.

Some success was achieved during the year in isolating the toxins from Chrysora. The crude toxin is a mixture of primarily two proteins, which have been separated from each other. Only one of the proteins (P_1), high in acidic amino acids, duplicates the neural toxicity of the whole toxin, whereas both proteins affect muscle action. By use of desheathed frog sciatic nerve, Dr. Blanquet has found that P_1 decreases the action potential of the nerve upon exposure for as little as two minutes. Subsequent exposure for an additional two minute period causes a further drop in action potential. These experiments have clearly established the nerve-blocking

action of the toxin. In contrast to other nerve toxins, however, the protein does not bind to the nerve sheath or damage the nerve membrane but must be continually present in excess to produce its effect. This suggests an effect on membrane permeability to sodium ions or on the sodium-potassium ATPase pump. These aspects of the problem are currently being studied.

The simultaneous action of the toxins on both nerve and muscle fibers would appear to account for the extremely potent paralytic action of the toxins following medusa stings. Experiments with rat erythrocytes indicate that neither protein is hemolytic.

Phytotoxins. Dr. Benjamin Wilson and his colleagues at Vanderbilt University have continued their studies of the lung edema factors (LEF) produced in sweet potatoes infected with the fungus Fusarium solani. At least four toxins making up the LEF have been isolated and identified. In order of decreasing toxicity, they are ipomeanine, 4-ipomeanol, 1-ipomeanol, and 1,4-ipomeadiol. Each of the compounds has been obtained by chemical synthesis and their chemical properties determined. In addition to their lung edemagenic properties, three of the compounds are nephrotoxic in mice.

Preliminary histological studies on the lung reaction in mice to 4-ipomeanol have not revealed the sequence of events leading to intraalveolar, perivascular, and peribronchiolar edema and pleural effusion in this species. Thus further basic studies on pathogenesis are in progress.

Mycotoxins. Dr. William P. Carlton at Purdue University has done further work with several species of toxigenic penicillia, particularly P. viridicum, P. frequentans, and P. cyclopium. P. veridicum fed to mice caused lung tumors in about 65% of the animals. The tumors were classified as bronchiolar and alveolar adenomas and adenocarcinomas. In addition, the fungus produced a phototoxic syndrome in animals exposed to sunlight. The mice developed erythema, edema, and necrosis of the ears, and edema and erythema of the muzzle, paws, and tail. Keratitis and blindness occurred in some animals. Ocular lesions were confined mainly to the anterior portion of the eye, beginning as corneal edema and progressing to deep stromal keratitis with hypopyon and iritis.

Isolates of P. frequentans and P. cyclopium produced toxicity in mice characterized by ballooning degeneration of hepatocytes and nuclear hypertrophy in both hepatocytes and renal tubular cells. Several other toxic species were discovered, which produced primarily degenerative changes in the liver and kidney.

Using the frog as an experimental model, Dr. Barbara Joyce of Colorado State University, has provided for the first time evidence that toxigenic strains of Aspergillus flavus can synthesize aflatoxin and other toxic metabolites during simulated aspergillosis in an animal host. The implications of this are extremely important, should the fungus produce aflatoxins in homothermic animals at higher temperatures. Thus, Dr. Joyce is currently attempting to induce chronic aspergillosis in warm-blooded animals and to determine the possible consequences in terms of aflatoxin production and carcinogenesis.

Work has progressed with the Aspergillus ochraceus carcinogenic metabolite ochratoxin. Methods for purifying and for radiolabeling Ochratoxin A have been worked out by Dr. Russel Sinnhuber at Oregon State University and Dr. Herman Meisner at Duquesne University. In work with the purified compound, Dr. Sinnhuber has found that hepatomagenesis can be induced in the rainbow trout through exposure of the eggs for only 1 hour. A 50% incidence of hepatoma occurred 10 months after hatching. This is one of the few demonstrated instances of cancer induced in the embryonic state and the first time using fish.

In other related activities, Dr. Sinnhuber and his colleagues have been able to synthesize the naturally-occurring malvalic and sterculic acids, which act as cocarcinogenic agents in aflatoxin carcinogenesis. This has enabled him to conduct quantitative studies on the reactivity of the 1,2-dialkylcyclopropene moiety. Using purified material, he has shown that the compounds produce a number of biochemical, physiological and pathological disorders which may play a role in cocarcinogenesis. He is now in a position to explore the more fundamental parameters of cocarcinogenesis on a quantitative basis with purified material, including the stage of liver damage at which the liver is most susceptible to cancer formation. These studies should provide further insight into the basic phenomena leading to carcinogenesis.

Miscellaneous Carcinogens. Support for several studies on the etiological role of other potential carcinogens was continued during the past year. Of particular interest were studies conducted by Dr. Marvin Friedman at the Medical College of Virginia on nitrososarcosine as an environmental hazard. Dr. Friedman has shown that when dimethylglycine is incubated with nitrite at 37°C (body temperature) there is significant production of nitrososarcosine. Moreover, gastric synthesis of nitrososarcosine was simulated by stomach deposition of dimethylglycine and sodium nitrite in the mouse. Since both of these substances may occur in foods, either as natural components or preservatives, the possible carcinogenesis of nitroso derivatives of natural amines and amino acids is of great concern.

In further studies, Dr. Friedman found that about 20% of mice fed nitrososarcosine developed squamous cell carcinoma, lung or liver tumors. Feeding of sarcosine and sodium nitrite induced primarily lung tumors.

Tests were conducted also on the mutagenicity of nitrososarcosine. Using the host mediated assay, the mutation frequency was increased about 10-fold. No evidence of mutagenicity was obtained with the dominant lethal assay. Combinations of nitrite and amines were also weakly mutagenic in the host mediated assay tests.

These findings represent the first demonstration of a significant carcinogenic response upon simultaneous feeding of sodium nitrite and a naturally occurring amino acid, and they underscore the potential hazard of gastric nitrosation of amines resulting from use of nitrites as food preservatives.

Dr. Robert Chang of the University of California has attempted to develop a more rapid method for testing the potential carcinogenicity of environmental

agents. He has devised a test employing induced transformation of cultured human cells to a form with heritable increases in growth rate and life span. Although few positive results have been obtained for several environmental agents implicated in malignancies, he has discovered an unexpected phenomenon associated with mononucleosis. Mononucleosis patients excrete a filtrable agent with the capacity to transform human leucocytes into rapidly and persistently multiplying lymphoid cells. By several tests, Dr. Chang has established that the agent, while viral in nature, is not the Epstein-Barr virus, and postulates that it is a heretofore unknown virus. The importance of the finding consists in the fact that this is the first demonstration of the shedding by man of cell transforming agents into his environment.

Physical Factors

The influence of physical factors in the environment on the human body have long been recognized. The effects of light, both visible and ultraviolet, in circadian rhythmic processes and in cancer of the skin are recognized but still poorly understood. More recently, there is growing recognition and concern about the effects of noise, microwaves, laser radiation, and radioactive nuclides and their interactions with chemical and infectious agents and disorders of genetic origin.

Heat. For apparently the first time, Dr. Barbara Drinkwater and her colleagues at the University of California's Institute of Environmental Stress are attempting to assess heat stress in women in relation to hormonal and related physiological changes during the menstrual cycle. The studies include females at all stages of life from prepubertal through post-menopausal. Though the studies have been underway only a short time, some physiological changes related to the menstrual cycle and heat stress have already been observed. These include lower Na^+ and Cl^- losses in total body sweat during luteal phase heat exposures than during other menstrual phases.

Other studies indicate the importance of conditioning. Significant improvements were found in ventilatory and cardiovascular capacities following periods of submaximal work, suggesting that ways may be found to minimize the effects of heat stress, which may take on greater significance during specific phases of the menstrual cycle.

Dr. Pharo Gagge and his colleagues at the John B. Pierce Foundation in Connecticut have continued research to provide a basic understanding of man's response to his thermal environment. Much of the effort has been devoted to physical characterization of the thermal environment in terms of a useful index of heat stress and of heat strain. It was shown that there is a quantitative relationship between skin temperature and internal body temperature in the regulation of body temperature by sweating during both rest and exercise. Changes in temperature within the hypothalamous were found to influence behavior, and relationships between behavior and the sensation of temperature and pain from the skin due to radiant heat have been quantitated in regard to the duration, intensity and spatial changes. The ultimate objective of these studies is to determine the quantitative relationships between the voluntary and involuntary actions of man in

response to heat in order to determine optimal conditions for rest, work, and a number of more subtle physiological functions.

Light. In a basic study of physical factors on the endocrine system, Dr. Richard Wurtman and his associates at MIT have continued to explore the influence of light on hormonally-controlled processes in mammals. The studies are concerned with the basic molecular events through which neuroendocrine effects are initiated and carried out. Their findings constitute probably the first available information on the action spectrum for any nondermatological or non-visual effects of light on intact animals. They indicate that the photopigment which mediates the pineal response to light, and perhaps other neuroendocrine effects, is rhodopsin or a similar compound. Since rhodopsin is the photopigment of rod cells, and rod cells are the only photoreceptor cells in the rat retina, the data suggest that the separation of the photic input into that concerned with vision and that responsible for other physiological effects occurs not within the eye but solely within the brain. Thus retinal pathology that occurs in continuous light does not block the ability of the eye to function as the portal of entry for at least that portion of photic input concerned with neuroendocrine functions.

Further studies on the pineal enzyme hydroxyindole-O-methyl transferase (HIOMT) have been carried out. HIOMT enzymes from the retina and harderian gland, which are concerned with control of the daily rhythm in body temperature, have been characterized with respect to biochemical properties and compared with the pineal enzyme. It appears that the retinal and pineal enzymes are similar but differ from the harderian gland enzyme.

In addition to control of body rhythms through the endocrine system, light was shown to have an influence on the acute toxicity of certain natural toxins, such as aflatoxin and monocrotaline (hepatotoxins). In the case of aflatoxin, either light or riboflavin will enhance acute toxicity, and when the two are combined there is further enhancement. On the other hand, carotene appears to inhibit aflatoxin hepatoxicity, with or without light. Monocrotaline toxicity is inhibited by light, and light plus riboflavin has a marked protective effect against its action. These results indicate the complex nature and the potential importance of light in the action of sensitizing food ingredients in biological systems.

Studies involving laser radiation are being carried out by Dr. Karl Weiss at Northeastern University in Boston. Laser radiation has potentially significant therapeutic applications, but it is also a health hazard. Dr. Weiss' research is concerned with establishing an understanding of the mechanisms and consequences of laser excitation at the molecular level. A satisfactory laser has been designed specifically for multiphoton experiments with flavins and visual pigments considered to be of great physiologic importance. In experiments with flavins, it was found that alloxazine, lumichrome, and riboflavin all give rise to fluorescence when subjected to pulsed laser radiation at 694 nm, whereas none show absorption in ordinary light. The experiments demonstrate that laser radiation can produce radicals from natural components which do not normally form as a result of other types of irradiation. It is suggested that such radicals may constitute the basis of laser radiation damage in cells.

Work was recently begun on the laser photolysis of visual pigments and related compounds. This should provide important insight into potential effects of lasers on rhythmic phenomena in view of the known role these pigments play in biological rhythms.

Odor. A project conducted by Dr. William Cain is directed at the biological consequences of odor pollution. Various aspects of the problem have been explored for both theoretical and practical importance. The sense of odor may serve, among others, as a warning device; and in his studies of trigeminal sensitivity, Dr. Cain has attempted to describe the properties and effects of air-borne irritants on response to odors. It was found that two odorants do not smell as strongly as the sum of the components smelled separately. An odorous substance can counteract the odor of any other substance, but the masking ability varies from substance to substance. As a generality, it appears that the sense of smell is impaired in an environment of complex mixtures and, therefore, loses a great deal of its usefulness as a measure of intensity of concentration of noxious substances, hence as a warning device.

PHARMACOLOGY-TOXICOLOGY

While the primary concern of the individual and the public centers around the overt manifestation of environmental diseases and mutational events, the tools of preventative and therapeutic action can best be provided by understanding of the fundamental principles and mechanisms by which biological systems function and by which functional changes are brought about. The purpose of this program is to provide such basic understanding. In the simplest terms, it deals with the questions of how and why toxic agents produce the recognizable disease endpoints. In practice it deals with an intricate web of interacting chemical, physical, and biological factors that affect, on the one hand, the transport, accumulation, transformation, storage, elimination, and inherent properties of chemical agents, and, on the other hand, the integrity and function of living systems at the molecular, organ and organismal levels.

Toxicology

A substantial number of studies were supported during the past year to provide further insight into the toxicological aspects of environmental agents.

Dr. Orville Privette of the University of Wisconsin continued his studies on the possible role of ozonides and hydroperoxides in ozone toxicity. He found that intravenous injection of ozonides and hydroperoxides in the rat produced marked lung edema and congestion. No overt damage to other organs was visible. Prostaglandins were released as a consequence of treatment in a manner analogous to their release in general tissue damage and apparently was not a specific response. These findings are in accord with the hypothesis that ozone damage to lung tissue is due to reaction of ozone with lipids to form ozonides or peroxides, which are presumably the agents directly responsible for tissue damage. An important aspect of such studies is the detection of molecular changes in target tissues prior to development of irreversible pathological changes, since therapeutic and/or prophylactic

measures at this stage offers the best opportunity for disease prevention or amelioration.

Dr. Arthur Aronson and his colleagues at Cornell University have provided further data relating the action of chelating agents to collagen degradation. This may have important implications in degenerative diseases such as emphysema. It was found that administration of calcium ethylenediaminetetra-acetate (CaEDTA) increases lipid peroxidative activity due to chelation of zinc in the liver, which results in labilization of cell and other membranes. Studies on lysosomes indicate enhanced lysosomal enzyme activity, and Dr. Aronson postulates that the liberation of cathepsin D from the lysosomes is particularly significant in the initiation of collagen degradation.

Studies on structure-activity relations of various polyaminocarboxylic acid chelating agents have led to some generalizations regarding the role of chelating agents in collagen degradation and other processes dependent upon metals. Preliminary data indicate that toxicity of the agents is related to the stability constant of specific chelate complexes, which is a function of both the chelating agent and the metal. However, more data are needed before definitive conclusions can be drawn regarding toxicity and specific disease processes.

Studies by Dr. Milos Chvapil at the University of Arizona have provided evidence that zinc plays a vital role in protection of tissues against toxic metals and gases and, by converse evidence, corroborates the studies by Dr. Aronson. He found that zinc ions and zinc complexes of 8-hydroxyquinolinol markedly stabilize lysosomes. Cd and Pb also added stability to the membranes although they were less effective than zinc. Studies of zinc distribution within the lysosomes indicate that the stabilizing effect occurs at the surface of the membrane and does not appear to be related to effects on known phospholipases. Studies on the plasma membranes of several types of cells, including those of alveolar macrophages and erythrocyte ghosts, show that zinc is present in all such membranes and linked to specific macromolecules. The protective action of zinc can be demonstrated in exposures to a number of toxic agents which are known to affect membranes. Among these are noxious gases and carbon tetrachloride. In some cases, at least, the protective action appears to be associated with the capacity of zinc to inhibit or limit the peroxidation of lipids in the membranes.

Heavy Metals. A substantial number of grants were supported on various toxicological aspects of heavy metals. Dr. Keith Byington of the University of Missouri has investigated the mechanisms of organotin toxicity in mammals. These compounds have found considerable use as pesticides in recent years but relatively little toxicological data in mammals is available as yet. The investigation provides evidence that organotins are capable of altering the permeability of biomembranes and that the effects are countered by certain thiols. There are also indications that the more lipid soluble compounds containing three carbon-tin bonds are very hemolytic in warm-blood animals and that they are highly specific inhibitors of mitochondrial oxidative phosphorylation, perhaps through their effects on the mitochondrial membrane structure. In general, the organotins show many of the properties of other lipid soluble organometallic compounds.

Further advances were made toward understanding the clinical, pharmacological and biochemical aspects of lead and mercury intoxication. Dr. Attalah Kappas of the Rockefeller University has examined the effects of lead on hepatic heme synthesis, cytochrome P-450 content and function, and the activities of the drug and hormone metabolizing enzymes in the mixed function oxidase system to which the heme protein is coupled. This system plays a predominant role in the oxidative transformation of drugs and other foreign chemicals in the liver. Thus, impairment of heme synthesis in the liver comparable to that known to take place in bone marrow in plumbism would be expected to make lead-burdened individuals susceptible to intoxication by a wide spectrum of drugs. In the course of studies with lead poisoned children, it was found that children in the age range 2-7 years metabolize drugs (antipyrine and phenylbutazone) via the oxidative P-450 pathway at least twice as fast as do adults. The children approach the adult rate of metabolism only as they enter the pubertal period. In children with frank plumbism, there was clear impairment of hepatic P-450 function both with respect to drug transformations and androgen metabolism. In some cases impairment was of sufficient magnitude to present a hazard in relation to endogenous hormone burden, use of therapeutic agents, and exposure to certain environmental agents against which the microsomal oxidases normally afford protection. These findings and their implications are currently being explored further.

Dr. Leon Golberg and his colleagues at Albany Medical College have continued research on a number of environmental chemicals with the primary goal of gaining a deeper understanding of the effects of such agents at the cellular and subcellular levels. The ultimate goal is to improve the predictability of toxicological findings in animals as indicators of hazards to man. The compounds under study include ethylene glycol, monosodium glutamate, cyclamates, saccharin, lead particulates, and organophosphate insecticides. Through conventional toxicological testing, indicated levels of human safety have been established with regard to the intended applications of the compounds and have provided further information about comparative toxicology.

At the subcellular level, studies on the action of 2,6-dichloro-4-nitroaniline (Botran) have revealed some striking effects of the compound on mitochondria and oxidative phosphorylation. Further studies on the use of mitochondria as indicator systems to reflect the toxicity of ethylene glycol and its metabolites and selective toxicity of fluorocarbons were carried out with promising results. Studies are currently in progress to assess the toxicity and hazard of combinations of two or more agents which act at the same intracellular sites. At the same time, efforts are being made to relate biochemical effects seen in subcellular systems to overt toxicological effects at the organismal level.

New Lead and Mercury Chelating Agents. Poisoning from inorganic or organic mercury and lead results in part from the tenacity with which they bind to cellular sites. Thus Dr. Mark Jones at Vanderbilt University has evaluated a number of new chelating agents with low toxicity and high selectivity for the ability to protect against toxic and lethal effects of mercurials. The family of agents under study, the thioethers, show high selectivity for mercury and appear to have sufficiently low toxicity for ultimate use in human therapy with fewer side effects than agents now in use. Three types of thioethers have been tested, e.g., those containing (a) carboxylic acid

functional groups, (b) aliphatic or aromatic groups or (c) both S and N donor atoms. In preliminary studies in experimental animals, the chelators show both a low degree of toxicity and substantial antagonism towards the toxicity of methylmercuric chloride.

Metabolism and Toxicity Modifications of Environmental Agents

Numerous grant-supported studies are concerned with metabolism, activation or deactivation, and toxicity enhancement and quenching of a broad spectrum of environmental agents. Such studies are of utmost importance in understanding the ability of the body to cope with foreign chemicals and are related directly to the ability to make rational decisions with respect to environmental and body burdens vs risks to human health.

Dr. John Casida and his colleagues at the University of California, in studies under a program grant, have carried out perhaps the most extensive and comprehensive studies of any single laboratory on transformations of pesticides and other toxicants and on the toxicology of transformation products. The program is divided into 5 subprojects. The first deals with pyrethroids, both natural and synthetic, which are among the safest insecticides. Dr. Casida's laboratory has provided much of the present understanding of the mechanisms of degradation of these compounds, both in the external environment and in the body, and the biochemical basis of their remarkable toxicological selectivity in insects. The second subproject deals with degradation of chlorinated hydrocarbons and the toxicology of degradation products. It includes studies on the composition, metabolic fate and transformations of the components of toxaphene, the identification of some new photoisomers of methane-bridged cyclodienes, and chemical and metabolic studies on the extraordinarily toxic and teratogenic tetrachlorodibenzo-p-dioxin. The third subproject includes some old and some new pesticidal chemicals which have in common the ability to uncouple phosphorylation. This property is being investigated with regard to stereochemical features of the molecules. Of particular interest are rotenone and organotin compounds.

The fourth area of study is the degradative chemistry of organophosphates and methylcarbamates. These compounds are becoming increasingly important as replacements for more persistent organochlorine insecticides. The studies deal with elucidation of the metabolism and identification of intermediate and terminal products, the mechanism of the oxidation reaction, and with the synthesis and toxicity evaluation of metabolites and nonmetabolic degradation products. The mechanisms of teratogenesis and neurotoxicity of a new class of toxicants, cyclic phosphorus esters, discovered in Dr. Casida's laboratory, are being tested for toxicity in various mammalian and nonmammalian systems.

The fifth subproject is a very broad one which includes a diverse array of compounds. It is concerned primarily with finding new, safer and more effective ways of controlling pests. It includes work on insect juvenile hormones and their analogues, a variety of thiocarbamate herbicides and a large number of potential insecticidal and fungicidal compounds. Thus it involves many classes of organic compounds, including persistent and non-persistent ones, some that are highly toxic to mammals and some that are relatively nontoxic but show desirable specificity toward a variety of pests.

Work at Oregon State University by Dr. Leon Terriere is primarily concerned with insecticide detoxication mechanisms. Using insects as models, Dr. Terriere has demonstrated that the microsomal oxidases, as well as DDT-dehydrochlorinase, two dissimilar enzyme systems, are induced 2 to 20-fold by a variety of compounds, including organochlorine insecticides, phenobarbital, and certain natural and synthetic insect hormones. Induction is generally greater in resistant than in susceptible insects. This suggests that resistant insects possess more genes for detoxification systems than the susceptible ones. This premise has led to major efforts to unscramble genetic factors involved in different types of insecticide resistance and to determine the associated biochemical parameters of resistance. A three-chromosome study of a naphthalene-resistant strain and two five-chromosome studies of a carbamate and a DDT-resistant strain to produce substrains with different chromosomes from the resistant parent have been carried out. In some cases a single chromosome appears to carry all factors necessary for high oxidase level, but in others two chromosomes are necessary for maximum activity.

Dr. Sheldon Murphy of Harvard University has studied the factors affecting the activity of a number of pesticides. He found that triorthotolyl phosphate (TOTP) inhibits both the catalytic hydrolysis of the carboxy ester linkages in malathion and malaoxon and the binding (hence inactivation) of malaoxon to tissue constituents. This explains the increasing potentiation of the insecticides by increasing doses to TOTP. Potentiation after low doses of TOTP appeared to be related primarily to inhibition of catalytic activity and binding to carboxylesterase. At high doses of TOTP, competition with malaoxon for binding sites on liver pseudocholinesterase appeared to be an important mechanism.

Studies such as these, which provide basic understanding of the chemical and biological factors that influence the susceptibility of vertebrates to poisoning by insecticides, are expected to be useful in prediction of conditions of susceptibility from species to species and in the development of new pesticides.

Studies by a number of other investigators have facilitated further progress toward this goal. Dr. Robert Neal and his colleagues at Vanderbilt University, using a series of parathion analogues and $^{18}O_2$ and $H_2{}^{18}O$, have provided convincing evidence that the mixed function oxidase catalyzed metabolism of parathion proceeds to the toxic metabolite paraoxon and the non-toxic metabolite diethylphosphorothioic acid. Studies with different animal species reveals a wide difference in the ability of different species to carry out the reactions, making it dangerous to generalize about the degradative capabilities of each organism likely to be put at risk by parathion exposure.

Further understanding of the toxicity and fate of various chlorinated hydrocarbons and species differences with respect to susceptibility has been developed by Dr. James Allen at the University of Wisconsin Medical School. In work with PCBs, he has shown that there is a progressive enlargement of the liver of rats within 24 hours after treatment. Hypertrophy was found to be associated with proliferation of the smooth endoplasmic reticulum, development of large membranous concentric arrays and an increase in lipid droplets within the cytoplasm of affected liver cells. Similar results were obtained

with rhesus monkeys although there were some distinct differences between the two species. In the monkey, liver hypertrophy and increased activity of certain enzymes were observed. However, these changes were not as great as those observed in the rodent and were subject to more individual variation even though the rat tended to develop degenerative changes in the liver less rapidly than the monkey. Thus species differences in susceptibility are not necessarily reflected in degree of liver damage. When the data and observations obtained in these studies are compared with the rather extensive data available for human patients exposed to PCBs in the Japanese episode of 1968, it is readily apparent that the symptoms and lesions produced by the PCBs are much more similar in the rhesus monkey and man than in the rat and man.

Dr. Allen has also explored the distribution and fate of dibenzo-p-dioxin in the rat, using ^{36}Cl-labeled dioxin. He found that about 90% of the administered compound was excreted in the feces and about 5% in the urine. After a 7-week recovery period, almost all of the retained compound was in the liver and concentrated in the microsomal fraction, with about equal distribution between the smooth and rough microsomes.

This research presents new insight into the function of the hepatic endoplasmic reticulum (ER) in regard to retention of chlorinated aromatic hydrocarbons and enzyme modifications brought about by the compounds. It was clearly demonstrated that the modifications in the ER, such as enhancement of enzyme activity and modifications in its compositional makeup, are directly attributable to the chlorinated hydrocarbons. In addition, it appears probable that the lipid portion of the membrane may be responsible for the storage of hydrocarbons for a much longer period than any other organelle of the liver. Thus, the ER may deserve greater attention in attempts to understand and evaluate the hazards of chlorinated hydrocarbon than heretofore appreciated.

Tolerance development is frequently observed in relation to drugs and other foreign chemicals and is part of the defense system against chemical overload. It also plays an important, though incompletely evaluated, role in man's adjustment to his chemical environment. Dr. Wolf Dettbarn of Vanderbilt University School of Medicine has attempted to elucidate the basis of development of tolerance to organophosphate insecticides. The project is designed to cast light upon the relation between changes which occur in various neurophysiological and neurochemical systems during the development of tolerance. It is believed that these findings will be applicable in principle to situations in which man is subject to chronic use of drugs and exposure to a wide spectrum of environmental agents. He finds that paraoxon induces release of acetylcholine which can give rise to several mechanisms of adaptation. One may be a return to normal acetylcholine release under repeated paraoxon exposure or adaptive increase in reuptake of acetylcholine. A third possibility is an adaptive decrease in receptor sensitivity. Studies on these possibilities are being pursued.

Dr. Dettbarn has found also that paraoxon partially inhibits a mechanism that removes Ca^{++} from the inside of sarcoplasm of muscle fibers and from axoplasm of nerve terminals. The block of the Ca^{++} pump has not been reported before and may be a possible explanation of organophosphorus action not solely accounted for on the basis of cholinesterase inhibition.

Various aspects of interactions between toxicants and other types of chemical agents including drugs were investigated by Dr. Virgil Freed and his colleagues at Oregon State University. They have examined the effect of chronic DDT exposure on pentobarbital tolerance in the rat. Three consecutive daily intraperitoneal doses of pentobarbital were shown to produce a significant tolerance in naive rats, with sleep times significantly shortened on both day 2 (to 65% of control) and day 3 (to 53%). This was correlated with a significant increase in the in vitro rate of hepatic microsomal pentobarbital metabolism (235% at day 3). On day 23 (after 20 days of barbiturate abstinence) the animals exhibited an intolerance (sleep time = 126%) not associated with any change in hepatic enzyme activity. All parameters returned to control values by day 43. These results suggest that the intolerance is nonhepatogenic and is probably related to enhanced CNS sensitivity. In parallel studies, chronic dietary exposure to technical DDT caused increased liver enzyme activity which did not prevent the appearance of tolerance by day 3 but did prevent the appearance of intolerance at day 23. The hepatic induction produced by DDT appears to offset any CNS sensitivity associated with delayed barbiturate intolerance.

Studies on the interactions between organochlorines and CCl_4 show that exposure of rats to low levels of DDT greatly potentiates the hepatotoxicity of carbon tetrachloride (CCl_4), as demonstrated by increased serum transaminase levels, altered liver function tests and histopathologic changes. Doses of CCl_4 up to 1 ml/kg produced only mild to moderate hepatic damage, yet a low dose of CCl_4 superimposed on a low dietary level of DDT resulted in greatly elevated activities of 2 serum transaminase enzymes as well as moderate-to-severe hepatotoxic lesions. DDT had little effect on the bromosulfophthalein retention test of liver function, while CCl_4 produced only mild hepatic function impairment by itself. The same dose of CCl_4 administered to DDT-fed rats produced a 7-fold greater impairment of liver function. Studies over a 48-hour period demonstrated close correlation of serum enzyme levels, liver function tests and histologic hepatocellular damage. Compartmental analysis of blood and liver concentrations of CCl_4 over a 48-hour period indicated that the potentiation could not be explained on the basis of enhanced partitioning of CCl_4 within the livers of the DDT-fed rats.

Spinal cord transection protected the rats from CCl_4 hepatotoxicity whether given DDT or not. After spinal cord transection, the body temperatures of the rats fell to 25°C, the drug-metabolizing ability of liver fractions from control and DDT-fed rats was reduced, and the level of cytochrome P-450 was not changed.

In further interaction studies both Pb and lindane were found to depress the hematopoietic system of the mouse. Studies were undertaken to determine the effects of combinations of Pb and lindane on the synthesis of hemoglobin and cytochrome P-450. The concentration of hemoglobin in the blood was decreased by Pb but was unaffected by lindane given alone. Given with Pb, lindane prevented the decrease. Lead also depressed levels of hepatic microsomal cytochrome P-450 by as much as 50%; lindane had the opposite effect and abolished the effect of Pb. Both Pb and lindane elicited slight increases in urinary excretion of coproporphyrin III: the effect of coadministration was additive. Lead brought about an increase in urinary excretion of

δ-aminolevulinic acid, whereas lindane had no effect on this parameter when given alone, but added to the increase produced by Pb.

Interaction between dietary methionine and DDT metabolism was also studied. Protein malnutrition is perhaps the most widely spread nutritional stress; therefore, some studies were initiated to evaluate the influence of a protein low in methionine (α-protein of soybeans) on the biological response to DDT. With rats raised on rations containing this protein as the sole source of nitrogen, DDT depressed growth and interfered with vitamin A utilization. Supplements of methionine counteracted the DDT effect.

It was also possible in this study to observe the influence of methionine level on the metabolism of DDT. The concentrations of DDT and its two primary metabolites, DDE and DDD, in liver tissue were determined. Under the conditions of the experiment, DDD concentration was not affected by the dietary methionine. The concentrations of DDT and DDE in liver increased with increasing levels of dietary methionine, with females showing the more pronounced response. It has been suggested that the response to dietary methionine could involve an effect on lipid transport.

Phthalate Esters. The biological and toxicological evaluation of phthalate esters was continued by Dr. Robert Rubin and his coworkers at Johns Hopkins University. This laboratory previously discovered a significant migration of phthalate plasticizers from polyvinyl biomedical devices such as blood storage bags and tubing used in cardiopulmonary bypass and renal dialysis units. A subsequent study of autopsy tissues of humans who had received blood transfusions revealed significant levels of di(2-ethylhexyl) phthalate (DEHP) in a variety of organs. The toxicologic effects of phthalates on several biological systems have been carried out and were reviewed in last year's report. Of considerably more importance from a longer point of view have been an increasing number of reports over the past year of the detection of phthalate esters in the environment. They have been found to varying degrees in the Great Lakes, the Mississippi River, the Charles River in Massachusetts, in the bottom sediment of portions of the Gulf of Mexico, etc. Certain waters have 10-fold greater contents of phthalates than of DDT, dieldrin or aldrin.

This widespread environmental distribution of phthalate esters has been confirmed by the detection of metabolites of the phthalates in the urine of humans. In addition, during the past year, tissues from approximately 150 autopsy cases, complete with histories of exposure to plastic biomedical devices have been received from hospitals across the country. The tissues are being analyzed for DEHP content. This study is being carried on in collaboration with Baxter Travenol Laboratories of Morton Grove, Ill. Preliminary results indicate that DEHP can frequently be found in the tissues of transfused humans.

During the past year, a major portion of the effort in this area has been devoted to a study of the adsorption, distribution, metabolism, and excretion of the compounds in experimental animals. In the intact animal considerably greater rates of metabolism and excretion were seen than might have been

expected on the basis of previous liver experiments. Following an intravenous dose of DEHP, the blood is rapidly cleared, with a half-life of 5-9 minutes. Thus, within 1 hour after an IV injection only trace amounts of the DEHP can be found in the blood. At that time, most of the injected dose can be found in the liver. The remainder is found primarily as water-soluble metabolites in the urine or feces. After 24 hours, the DEHP has essentially left the liver and the total dose can be accounted for almost entirely as metabolites excreted in the urine and feces. Following an oral dose of DEHP, the total dose can be accounted for almost entirely as metabolites in urine and feces. These results strongly suggest that the absorption of DEHP is rate-limiting relative to metabolism and excretion so that as soon as DEHP is absorbed, it is readily metabolized and excreted.

Aflatoxin Metabolism. Further studies on the toxicity and metabolism of the important fungal metabolite aflatoxin were carried out in several laboratories. Dr. Colin Campbell of Virginia Polytechnic Institute has attempted to determine if the pathways of metabolism and mode of excretion of aflatoxins by humans are the same as those in other mammals. It has been shown that the O-demethylated derivative of aflatoxin, designated AFP, is a major metabolite and excretory product of rats and monkeys receiving aflatoxin. Although production of very small quantities of the metabolite could not be ruled out due to the necessity of limited intake, no evidence was found for the derivative in the urine of human subjects known to have consumed quantities of aflatoxin B_1 sufficient to produce a previously characterized metabolite, aflatoxin M_1. The methodology used was capable of detecting the free phenol or the sulfate or glucuronide conjugate. The findings, therefore, suggest that human metabolism of aflatoxin probably differs from that of the rat or monkey.

Continued studies on the mechanism of aflatoxin carcinogenesis indicate that some type of metabolic activation seems to be required for maximal effect. This is reminiscent of, and seems to parallel, the activation of benzpyrene and other carcinogenic polynuclear hydrocarbons. The site and mode of action in tumor induction remains to be elucidated.

Detoxication of Metals. Despite the fact that heavy metals are not destroyed and are often retained in the body, there is growing evidence of effective, inducible mechanisms for detoxication of development of tolerance to the various toxic ions. Dr. Albey Reiner at the University of Massachusetts, using bacterial systems, has attempted to develop further understanding of protective devices available to living systems at the cellular level. Studies during the current year have centered around the isolation of *E. coli* mutants resistant to cadmium, mercury, and silver ions and studies of the resistance mechanisms in such mutants. Approximately 100 mutants have been obtained. They grow well at cadmium concentrations approximately 10-fold greater than those tolerated by the wild type. In contrast, no mutants resistant to mercury or silver have been obtained as yet. These studies, while not yet definitive in terms of molecular changes, suggest that cells can be adapted to tolerate high levels of certain toxic metal ions. Such cells might serve as valuable tools in establishing a basis of understanding of the well-known differences in organ and tissue differentials in response to toxic metals through understanding of basic differences in individual cell types.

Another approach to this problem has been taken by Dr. Sara Bryan at Louisiana State University. Her studies focus on the induction of metal binding proteins, particularly metallothionein, and the localization of metals in subcellular components, such as chromatin, following exposures of mice to sublethal concentrations of cadmium, copper, and mercury. Results thus far indicate that both cadmium and mercury elicit increased production of metal binding proteins. The synthesis mechanism appears to be triggered 48 to 96 hours after treatment. Metallothionein appeared to be stimulated to a somewhat greater extent by cadmium than by mercury. Efforts are being made now to isolate and characterize the protein and to develop suitable immunological methods for its detection in tissues and cells.

Progress was made also in isolation and characterization of mercury and copper-binding chromatin. Preliminary studies show that challenge with mercury or copper results in increases of heterochromatin materials which bind up to 10-fold the normal quantities of the metals. Further studies will be conducted to determine the nature of the substances and their basic roles and mechanisms in metal tolerance.

Similar studies by Dr. Donald Buhler at Oregon State University indicate that metallothionein is probably only one of several important proteins in metal binding and detoxication. Using California sea lion liver and kidney tissues, four mercury-binding proteins have been found, only one of which appears to be metallothionein. Work is in progress toward further characterization of the other proteins.

PATHOGENESIS

Because of the extraordinarily wide range of chemical agents in the environment, no organ or body function can be considered to escape entirely the adverse influences of environmental toxicants. Environmentally-related diseases of the pulmonary and cardiovascular systems, the liver, kidneys, reproductive system, digestive tract, central and peripheral nervous systems, and degenerative diseases of complex and long-term etiology are well-documented. However, the mechanisms and manifestations of malfunctions and structural disorders are just beginning to be clarified. Knowledge of initial or early molecular and cellular lesions and understanding of their role and time course in pathological endpoints offers the best hope, aside from prevention of exposure, of detecting and countering pathogenic processes before damage becomes irreversible. The objective of this program is to develop such knowledge and understanding.

Neuropathology

Many environmental agents of primary concern have been shown to affect the central and/or peripheral nervous system and behavioral patterns in experimental animals. Among these are various insecticides and heavy metals. Thus a number of projects supported through the grants program have focused upon the pathology and consequences of these effects.

Insecticides. Dr. Robert Jay of the University of California has established that DDT and dieldrin produce changes in the CNS, culminating in convulsions

at high doses. Through analysis of evoked responses, he has shown that a major effect of dieldrin is to selectively increase cortical responses to sensory, transcallosal and direct cortical stimuli. Massive cortical cell discharge accompanies the enhancement of evoked responses and the sensorimotor cortex is made hyperexcitable. A number of other chlorinated hydrocarbons appear to be similar in their convulsive properties, but some differences were found in the time required for the onset of convulsions. This may be associated with rates of distribution in the body and penetration into the CNS.

Dr. Dorothy Woolley, also of the University of California, has conducted somewhat similar studies in rats on the organophosphate and carbamate insecticides, beginning with effects in utero. She found that pups born to parathion-treated dams exhibited delayed development of the startle reflex, eye opening, and the righting reflex. Brain cholinesterase activity was normal. Thus, the effects may be due to poor nutrition resulting from reduced lactation of the mother and/or abnormal behavior toward the suckling pups. However, effects directly on the fetus were not ruled out.

She also studied the effects of parathion on acetylcholinesterase (AChE) and pseudocholinesterase (ChE) activities in the blood and plasma in the bonnet and rhesus monkeys and attempted to relate them to performance of learned visual discrimination tasks. At parathion dosages that reduced the activities of both enzymes, performance of the tasks was reduced and remained below pretreatment levels for several weeks before returning to normal.

These and other experiments in mice show clearly that behavior is indeed affected by parathion at doses which do not cause other symptoms of poisoning and that the effects are completely reversible after short exposures.

Dr. Virgil Freed and his colleagues at Oregon State University have also observed DDT-induced modifications in responses of animals to barbiturates. Of note are changes in brain function. Barbituates are widely used as anti-convulsant drugs and DDT has the effect of lowering the efficiency of the compounds in this respect. Further work is in progress to determine the basis and site of interactions of the two compounds in the CNS.

Lead. Several studies of CNS effects by heavy metals have provided additional insight into acute and chronic syndromes. Dr. Robert Feldman of University Hospital in Boston has conducted a study of the effects of lead on nerve conduction velocity in children. The data show that there is a slowing of conduction velocity as blood lead levels greater than 40 mg/100 cc blood, and that the effect has continued in known cases of plumbism for as many as 2 to 25 years after poisoning. Other studies on the relationship between nerve conduction velocity and duration of lead exposure show that there is a progressive decrease in conduction with time.

Basic studies by Dr. Richard Manalis at the University of Cincinnati and by Dr. A. M. Goldberg at Johns Hopkins University indicate that some of the neural manifestations of lead toxicity can be explained by effects on the synapse. Dr. Manalis finds that lead affects the release of transmitter from nerve endings in two ways. First, it increases the frequency of

spontaneous or resting release of acetylcholine, the neurotransmitter from motor nerve terminals. Second, it concomitantly inhibits the phasic release of the transmitter following arrival of a nerve impulse. This dual action clearly demonstrates that the two processes occur by different mechanisms, since lead increases one and decreases the other. The effects are very rapid and are easily reversed. Thus they do not appear to account for the long-term neural and behavioral effects of lead.

The long-term effects would appear to be more closely related to pathological changes at cellular and subcellular levels, as elucidated in part by Dr. Werner Niklowitz at the University of Cincinnati. Dr. Niklowitz finds that after a convulsive dose of lead administered to the rabbit, there are significant alterations in components of pyramidal and astroglial cells in the brain. These changes can be seen before the onset of convulsions. In general, two types of degenerative changes occur, e.g., cellular sclerosis and cellular hydrops, each specific to certain cell layers of selected areas of the brain. Ultrastructural alterations in cells undergoing hydropic degeneration exhibit folding of the nuclear membrane, swelling and severe lesions of mitochondria, hypertrophy of the Golgi complexes and patchy chromatolysis of the Nissl substance (ergastoplasm). Progressive hypertrophy of Golgi saccules and vacuolization of ergastoplasm areas lead to degeneration of affected nerve cells. It is thought that these changes may be the basis of the irreversible and lasting effects of lead poisoning since affected cells are replaced slowly if at all.

Scientists at Oregon State University have extended their observations on neural effects of organochlorine insecticides and lead to consideration of combinations of the neurotoxins. Although no synergistic interaction between lead and organochlorines was noted in the case of epileptiform seizures induced by lead, concomitant exposure resulted in an increase in muscular incoordination characteristic of pesticide effects. Moreover, while lead exhibits only slight effects on motor performance, which is strongly affected by the pesticides, lead potentiated this effect when given in combination with DDT, dieldrin or lindane. Interaction was evident at very low doses of the compounds. Even nontoxic doses of DDT or dieldrin were made quite toxic upon administration of lead.

In addition to the well documented role of lead and mercury in behavioral and CNS aberrations, Dr. Chester Wilpizeski at Thomas Jefferson University has presented evidence of effects of these substances on auditory and vestibular processes. In chronic effect studies on the squirrel monkey, he has shown that though lead-induced threshold hearing shifts are fairly small in absolute terms, there is a reduction in the amplitude of evoked responses in auditory nerves as lead poisoning progresses. In general, no auditory or vestibular changes are seen during the first month of treatment but appear in increasing magnitude at 2 to 3 months. Experience suggests that longer term exposure will result in further and more drastic changes and studies are in progress to verify this experimentally.

Zootoxins. Protein neurotoxins of natural origin comprise perhaps the deadliest substances known, e.g., botulinum and tetanus toxins and the snake venom γ-toxins. In view of the substantial problem of snakebite in the United

States each year, the basic proteins and the mechanism of action of Elapid venoms are being studied by Dr. Robert Neal and his colleagues at Vanderbilt University.

The family Elapidae includes the cobras, mambas, kraits, and coral snakes; the latter being prevalent in the extreme southern portion of the United States. The venoms of these snakes are comprised of a specialized group of toxic basic proteins which act upon the nervous system and induce respiratory failure and cardiac arrest. The research in Dr. Neal's group (mainly by Dr. DiMari) is concerned with elucidation of the mechanisms by which the proteins act.

Two types of α-toxins from the Elapids have been isolated and tested: Type I, containing 61-62 amino acid residues cross-linked by 4 disulfide bridges [Cobratoxin from the Formosan cobra (Naja naja atra) and α-toxin from the spitting cobra (Naja nigricollis)] and Type II, containing 70-72 amino acid residues cross-linked by 5 disulfide bridges [α-toxin from the Indian cobra (Naja Naja Naja) and α-bungarotoxin from the krait (Bungarus multicinctus)]. The following observations have been made: (1) Type I induces an immediate but slow rate of decrease in diaphragm contraction whereas Type II elicits a delayed response which, once initiated, inhibits contraction at a faster rate than that observed with Type I, (2) reduced neurotoxins of both types are inactive, (3) from experiments in which reduced material is allowed to reoxidize slowly and is tested at various stages of oxidation, it appears that the formation of all 4 or 5 disulfides is not essential for toxic action. Toxin derivatives in which specific amino acid residues have been chemically altered are presently being prepared and will be tested using the diaphragm system.

The most basic of the venom proteins are the cytotoxins ("cardiotoxins"). In structure they resemble Type I neurotoxins. They are the agents believed to be responsible for local necrosis and for cardiac arrest following Elapid envenomation. They also have been reported to lyse tumor cells at a faster rate than they act on normal cell types (erythrocytes, muscle cells, etc.) and for this reason they appear to be potential agents for the chemotherapeutic treatment of cancer as well as for chemical probes to study the membrane morphology of tumor and normal cells. The cytotoxins are lethal but the reported dosage necessary to destroy Yoshida or Ehrlich ascites tumor cells is approximately one-tenth that necessary to kill the mouse in which the cells are cultured (the neurotoxins are twenty times more lethal than are the cytotoxins). Interest in this area is primarily to determine which portions of the molecule and which amino acid residues are essential for cytolytic activity. The mechanism of action of these substances is being explored further with possible applications beneficial to man in mind.

Renal Disorders

Heavy Metals. Further studies have been carried out toward a better understanding of renal effects of heavy metals and the long-term consequences of such effects. Dr. Ernest Foulkes of the University of Cincinnati has provided further insight into the pathological effects of heavy metals, particularly mercurials and cadmium. In studies with organomercurials, he has identified

the functional lesion responsible for aminoaciduria. The lesion occurs as a result of changes in membrane properties of tubular epithelial cells, which line the lumen of the tubules where the carrier system responsible for reabsorption of amino acids is located.

In his work with cadmium, Dr. Foulkes finds much the same kinds of lesions as those produced by mercurials. Analysis of artery-to-urine transport times leads to the conclusion that the transport systems responsible for amino acid reabsorption are localized on the luminal membrane and that, as in the case of other heavy metals such as mercury and uranium, this is also the site of pathological action of cadmium. A second locus of action of cadmium was identified as the peritubular cell membranes. Here at least four separate carrier systems actively transport different amino acids. Cadmium rapidly and specifically depresses uptake of dicarboxylic compounds but has no effect on that of other classes of amino acids.

Pulmonary Disorders

Coal Dust. Scientists at the University of Cincinnati have conducted extensive studies on the pulmonary effects of coal dust and the role of specific components of the dust in the etiology and pathogenesis of pneumoconiosis. They have shown that there are quantitative differences in chemicals extractable from bituminous coal from mines in Pennsylvania and Utah. The most striking chemical differences are in nickel and iron contents, with the Pennsylvania coal being substantially higher in both. Correlates between the incidence of pneumoconiosis in miners in Pennsylvania and Utah show that the former is significantly higher, which is in accord with the high nickel and iron contents of the coal.

Follow-up studies show that both metals are more rapidly leached from coal dusts with serum or *in vitro* cell culture media at physiological pH than with water. Moreover, experiments with cultured cells show that extracts from Pennsylvania coal are substantially more toxic than those of Utah coal, which again parallels the prevalence of pneumoconiosis in the two areas. Toxicity to the cells correlates with the nickel content specifically and with the inhibition of cellular respiration and uncoupling of phosphorylation in cell cultures by equivalent amounts of added nickel salts.

Although these studies must be viewed as only suggestive, other data in the literature on nickel indicate that this metal is a potent sensitizing agent when inhaled. It appears, therefore, to warrant more intensive study, particularly as a pulmonary hazard.

More fundamental studies were carried on also on the effects of coal dust *in vivo* on macrophages in rats. The data show that there is a substantial increase in the number of macrophages during the first month of exposure to the dust. However, phagocytic capacity, bacterial phagocytosis in particular, is substantially reduced. After three weeks of exposure, the animals also showed extensive pulmonary disease, including pneumonia and pulmonary abscesses. In animals exposed to nickel oxide dusts, the same kinds of syndromes were apparent, and the alveolar macrophages were altered in number and appearance.

Other heavy metal dusts also induced pulmonary changes. Notable among these was cadmium, which induced interstitial fibrosis with concomitant thickening of alveolar walls and subpleural connective tissues.

Dr. Joseph Brain of Harvard University has conducted a series of studies concerned with pathogenesis in the lung. The work focused primarily on effects of iron oxides, coal dust, barium sulfate, glass fibers, chrysotile and crocidolite on alveolar macrophages of rats and hamsters. Some species were exposed also to fly ash, cigarette smoke, carbon particles, and triphenyl phosphate. He found an increase in the number of cells obtainable by lavage upon administration of coal dust, chrysotile or iron oxide. In some cases the increases occurred within 2 hours after inhalation of the particles. In general, however, the increase was maximal a day after the initial exposure and remained elevated after 3 days.

Determination of mitotic indices indicated that the increase in free cells was largely attributable to the release of preexisting cells from a macrophage pool rather than to proliferation of new alveolar cells, since the increase was seen only 4 hours after treatment--a period too short for appreciable cellular proliferation. Experimental confirmation of this was obtained by whole body irradiation with X-rays sufficient to block mitosis followed by administration of carbon particles. The numbers of recoverable cells was the same in the irradiated and non-irradiated groups.

Subsequent studies with iron oxide and other particles indicate that while the numbers of cells increase upon exposure, the phagocytic capacity of the cells is reduced and the bactericidal capacity of the lung is seriously impaired. This is in accord with the findings of the scientists at the University of Cincinnati in their work with coal dust.

Ozone. Further insight into the pulmonary damage and pathogenesis associated with exposure to ozone was provided by Dr. Donald Dungworth at the University of California. Several hours exposure of rats to 2 ppm ozone caused a 50 to 60% reduction of sulfur-containing reducing substances in lung, and a 25 to 40% reduction in oxygen consumption. In contrast, 1 to 3 weeks exposure to low concentrations attained in polluted air (0.2, 0.5 and 0.8 ppm) caused an increase of 30 to 45% in pulmonary tissue oxygen consumption and up to 30% increase in a number of specific enzymes involved in regulation of cellular metabolism. The increases in enzyme activities indicated the probable onset of adaptive changes.

Enzymes capable of breaking down lung tissue were found to increase in concentration in the lungs of rats exposed to 0.8 ppm ozone for 5 to 7 days. Special biochemical tests have shown that lipid peroxidation resulting in damage to cell membrane is probably a basic mechanism responsible for the effects. However, a protective enzyme mechanism was discovered whereby peroxides formed upon lipid peroxidation are detoxified via the glutathione peroxidase system. Some of the enzymes involved in this system were shown to be induced by as little as a 7-day exposure to 0.2 ppm ozone. Thus, the pulmonary response is extremely sensitive and may be important as an adaptive biochemical change.

Measurements of the rate of killing of bacteria within lung tissue showed that the bactericidal potency was lowered by 4-hour exposures to as little as 0.4 ppm ozone and nitrogen dioxide. Four-hour exposures to combinations of ozone and NO_2 show that the two compounds act independently as far as reduction of bactericidal activity is concerned. Seventeen-hour exposures, which were more relevant for man, however, demonstrated an additive effect of ozone and NO_2 in causing reduction in bacterial killing.

Pathologic studies in rats proved that exposure to as little as 0.2 ppm continuously for 7 days cause mild but unequivocal damage in and adjacent to the smallest terminal airways. The airways were found to be focal points of damage at all levels of exposure. Dead cells and debris accumulated in the airways, hydrolytic enzymes were released, and changes in lining cell populations were obvious.

Similar studies by Dr. Gustave Freeman at Stanford Research Institute have provided further information relating ozone and NO_2 exposure to the development of emphysema and chronic obstructive lung disease. He found that rats exposed intermittently for a lifetime (about 2 1/2 years) to about 15 ppm NO_2 developed emphysema, a chronic obstructive lung disease (COLD), and exhibited an attendant loss of oxygen-absorbing surface area in the lung. This resulted from destruction of tissue, with a loss of about two-thirds of the air sacs (alveoli). Stagnant air was retained in the enlarged, obstructed lungs following their removal from the chest. The main impact of injury initially was in the smaller airways that conduct gases immediately to and from the alveoli. These features resemble those in man and constitute a reasonable facsimile of human emphysema.

Ozone has proven to be approximately twenty times more injurious than NO_2 to the lungs of rats. It induced a similar type of disease but with certain significant differences. These are, mainly, a slightly deeper (more distal) position in the lungs of the initial injury and an early stimulation of connective tissue growth at the sites of injury. Considerable reaction becomes apparent within a few hours of exposure to less than 0.5 ppm--a concentration readily achieved at peak traffic hours in some urban areas. Cellular changes are obvious within four hours of exposure to about 0.9 ppm O_3, a level occasionally reached in heavy smog.

A mixture of O_3 and NO_2 at concentrations as low as 0.25 ppm and 2.5 ppm, respectively, caused definitive injury to the lining cells of the small airways in the lungs within 24 hours; and a mixture of each at the occasionally high ambient level of about 0.1 ppm induced an advanced, lethal degree of emphysema within two months of continuous exposure.

Polycythemia (an excessive concentration of red cells) developed in weanling rats exposed to O_3, as it did in those treated with NO_2. When growing or mature rats were exposed to NO_2, the size of red blood cells was reduced but numbers and durability of the cells increased. In contrast, rats exposed from birth to about 15 ppm NO_2 had an absolute as well as a relative reduction in the number of red cells, compared with unexposed rats of the same age. Moreover, the cells tended to be larger than normal, but the amount of ATP in the cells was reduced.

When the airways and air sacs were naturally exposed to as little as 2 ppm NO_2 during respiration, the usual rate and replacement of the epithelial cells was accelerated, reaching a maximum during the second and third days and returning to the pre-exposure rate within a week of continuous exposure. Accelerated division of certain cells of the alveolar walls and of nonciliated cells lining the walls of the small airways occurred to replace the injured cells. Light and electron microscopy revealed that, beginning about two hours after exposure to either 15 ppm NO_2 or to 0.5 ppm O_3, ciliated cells of the small airways and of the thin, flat Type 1 cells from the adjoining alveolar walls began to be sloughed. However, following short-term exposure and allowing time for replacement of the dead cells, the new cells that appeared were relatively resistant to the same concentrations of gases.

Lung Carcinogenesis

Studies conducted by a number of grantees at the Institute of Environmental Medicine of New York University and others have made it increasingly evident that induction of human lung cancer is multifactorial in nature and dependent upon long-term exposure to combined agents. Animal models have been developed which duplicate human bronchogenic carcinoma, and studies with these have shown for the first time that there is potentiation between benzpyrene and sulfur dioxide in tumor induction. This has a significant bearing on the recent decision to relax controls on the use of coal and high sulfur fuels during the energy shortage, since both of these produce high levels of soot and SO_2 upon combustion.

Studies conducted by this group on pulmonary effects of fiberglass plant dusts and polyurethane foam dusts revealed a number of associated changes in the lungs. With polyurethane foam dusts, both centrilobular emphysema and squamous cell carcinoma were seen. Other pathological changes included marked changes in capillaries, alveolar epitheleal cells and increases in the number and activity of macrophages.

A retrospective study of cancer of the lung among industrial workers exposed to bis(chloromethyl) ether since 1948 was initiated by the group as a followup to the establishment of the carcinogenic potency of the compound (see last year's report). Records of exposed workers in five companies in which the compound was manufactured or used extensively are being collected from industry files. Mortality and cancer incidence will be determined through Social Security records and death certificates and related to the magnitude and duration of exposure in order to develop a clear understanding of the possible hazard of the material to man.

Some potentially important new evidence regarding the role of proteases in tumorigenesis has been reported by Dr. Walter Troll at New York University Medical Center. As postulated previously the carcinogenic process in mouse skin occurs in two phases. The first is an irreversible process carried out by primary carcinogens such as dimethylbenzanthracene or β-propiolactone. The second (cocarcinogenesis) is carried out by irritants such as croton oil or its purified component phorbol myristate acetate (PMA). It was noted previously that PMA caused an increase in esterase and protease activity in mouse skin, beginning at four hours after application and still detectable

twenty hours later. This increase is inhibited by the synthetic protease inhibitors, tosyl lysine chloromethyl ketone (TLCK) and tosyl phenylalanine chloromethyl ketone (TPCK). The enzyme appears to be a sulfhydryl protease since d and l isomers of TPCK are equally active as inhibitors. This is a characteristic of sulfhydryl proteases such as papain. The general action of proteases may be the derepression of the genome by hydrolysis of histone repressors.

This year Dr. Troll has been able to demonstrate that progesterone, after application of estradiol in rat uteri, causes the hydrolysis of histones. This action is prevented by protease inhibitors and the competitive substrate protamine. By use of special techniques for protease assay, Dr. Troll has been able to show that these compounds specifically induce a protease. Subsequent studies of the effects of hormones directly on tumor promotion provide further evidence of involvement of a protease. It was shown that dexamethacone and related anti-inflammatory hormones inhibit the promoting action of phorbol ester, which correlates with their ability to suppress inflammation. Phorbol ester also lowers the level of cyclic AMP in mouse skin, but it appears doubtful that this is related directly to the promoting activity of PMA since neither TLCK nor TPCK affect the phorbol ester-induced decrease in cyclic AMP but both inhibit promotion. These studies would appear to have important implications in the prevention and management of human cancer.

Carcinogenic and Other Effects of Environmental Radiation

New techniques and information relating to the biological effects of sunlight and other sources of radiation were developed by Dr. Frederick Urbach during the year. It has become obvious that there has been a dramatic reawakening of interest in the biologic effects of light, due in part to a real concern about possible alterations of the earth's atmosphere with the resultant changes in the quality of light from the sun, and in part to the proliferation and recognition of the plethora of photoactive chemical agents to which man is being exposed. Furthermore the development of new, intense light sources for illumination and industry has raised questions regarding the effects of chronic exposure to spectra differing greatly from those found in nature. Finally, the possible usefulness of light for therapeutic purposes is being seriously reconsidered.

New lamps, optics, filters, automatic controls, monitors and calibration and measurement equipment for irradiation of experimental animals and man with light closely simulating that of natural noon sunlight, for extended periods of time,have been designed, built and tested. This equipment has permitted conduct of critical experiments in the field of phototoxicity (effects of chemicals and light on the skin) and photocarcinogenicity. The equipment has proved useful for diagnosis of light induced diseases of man and for treatment of certain diseases. In particular, the design of large field solar simulators has allowed simultaneous studies of various spectra on tumorigenesis and other photobiologic phenomena.

Investigations on the genetics, anatomy and skin physiology of various hairless animals (mice, rats, pigs) have resulted in the development of hardy, easy to breed animal strains exceedingly useful for photobiologic studies.

A newly-developed hairless mouse strain is much superior to the usual inbred Jax hairless mouse. Minipigs have become useful since their skin is indistinguishable from the skin of man in anatomy, physiology, sensitization, etc.

The widespread use of phototherapy for hyperbilirubinemia in infants poses both great hope and possible serious side effects. The development of new, spectrally narrow highly active lamps has reduced treatment time and increased speed of bilirubin destruction. However, there are serious side effects possible--particularly those on unprotected retinas in the presence of oxygen and possible effects on entrainment of biologic rhythms. These, and other unwanted effects such as reduction in riboflavin and glucose 6PD in serum, are being studied intensively.

The new light sources have aided in the development of test systems for possible phototoxicity and photoallergenicity of a variety of environmental agents, and prediction of effects in man is now possible. Skin cancer can now be induced reliably in animals using the new equipment, techniques and animal models. This facilitates studies of the probable effect of alteration of atmospheric ozone, and interaction of light and chemicals in photocarcinogenesis. These new techniques, for the first time, allow reliable investigations into the mechanisms and causes of cancers of the skin.

Extensive epidemiologic studies, performed in Philadelphia and with collaborators in Galway, Ireland, and Queensland, Australia, have shown the importance of genetic as well as environmental factors for the induction of skin cancer in man. The Celtic type, with fair, freckled skin, light eyes, easy sunburning and little tanning are the most predisposed individuals. Also, the ability to measure natural sunburn ultraviolet light has made possible new insights into the relationships of sunlight to skin cancer in man, particularly the effects that partial screening would have.

Finally, an ultraviolet light television system has been developed, which should make it possible to easily and inexpensively identify people who are particularly likely to develop skin cancer and other light induced skin changes early in life. This will make it possible to select out those most prone to skin cancer before serious skin damage has developed, and to instruct them in proper preventative measures so that the risk of developing skin cancer later in life will be very significantly reduced.

MUTAGENESIS AND REPRODUCTIVE TOXICOLOGY

The most profound and permanent danger associated with environmental pollution consists in changes in the human genome and in the capacity to produce viable, productive and intelligent progeny. Evidence accumulated through several decades leaves little question of the genetic hazard of many of the products and by-products of contemporary technology and of a substantial number of chemical agents of natural origin. Moreover, there is growing conviction among medical scientists that genetic damage probably contributes to inherent susceptibility to disease and may indeed by causal in the case of some types of cancer. The current program in this area, both through directed intramural research and through the grants program, is concerned with developing understanding of the nature of mutagenic and teratogenic agents and other compounds

that might affect developmental and physiological characteristics of man. A major effort is being made also to develop and refine methodology for evaluation of exposure to such agents in man.

Development of Methodology

Dioxins. 2,3,7,8-Tetrachlorodibenzo-p-dioxin, commonly called dioxin, is one of the most toxic and teratogenic synthetic substances known. In the guinea pig, the LD_{50} appears to be less than 1 ppb body weight. The compound is present as an impurity in many industrial chlorophenols and, because of its stability in lipids, it is likely to undergo biomagnification as it moves through the food chain into man's diet, as well as through industrial exposure. Because of its extraordinary biological activity, its hazardous threshold lies near the limits of detection of most conventional analytical tools. With this in mind, Dr. Matthew Meselson of Harvard University has developed a highly sensitive analytical method for quantitative studies on the compound in body tissues and fluids and in the environment. The procedure makes use of a screening, double-focusing mass spectrometer capable of detecting less than 0.5×10^{12} g (0.5 parts per trillion) of dioxin in food and tissue with good reproducibility. The lowest limit of detection reported previously by other procedures was of the order of 50 ppb.

This development extends the capabilities for quantitative studies well within the range of usefulness for toxicological determinations in tissues where toxicity may result from concentrations below 1 ppb. Equally important, it provides a basis for application of principles established in these studies to low levels of other compounds, since the method is suitable for a wide range of agents for which detection methods were previously inadequate.

Mutagenesis testing. Dr. William Lee at Louisiana State University has continued to explore mutagenicity test systems with quantitative relevance to man. A major problem in meaningful mutagenicity testing is to place chemical mutagenesis on a quantitative basis in a eukaryote, reasonably representative of man, that can be reliably and economically analyzed genetically. Dr. Lee, by using combinations of tritium-labeled ethylmethanesulfonate and ^{14}carbon-labeled DNA in sperm cells of the fruit fly Drosophila, has been able to increase several-fold the sensitivity of methodology for quantitating alkylation at the 7-position of quanine, which is a measure of mutagenic potential of alkylating agents. The methods of dosimetry used should permit meaningful interpretation of negative results from genetic tests because the number of alkylations per genome that fail to give an increase in the spontaneous mutation rate can be determined. Since determinations are made directly on DNA, the method eliminates from consideration factors such as penetration into the germ line or metabolism of the chemical. By making comparisons between various species based on dose given versus dose measured as alkylations per nucleotide of DNA in the gamete, it is possible to factor out differences in metabolism and other events among species. The ultimate goal is to calibrate the method, using the economical Drosophila test system, with the more expensive mammalian test systems and to develop a sound basis for extrapolating results to man.

DNA Repair. Mammalian cells are known to possess mechanisms for repairing DNA damage induced by ionizing radiation. It is not known, however, to what extent these repair processes may influence the ultimate survival of irradiated cells or their sensitivity to radiation-induced malignant transformation. This question is being investigated by Dr. John Little et al. at Harvard University. Mutant diploid cells have been isolated which are variant either with respect to X-ray survival characteristics or DNA repair capacity. DNA repair is measured by observing the rejoining of radiation-induced DNA strand breaks by use of alkaline sucrose gradient techniques. Two types of DNA damage and repair have been demonstrated: (1) an incisional process which probably represents the enzymatic recognition by the cell of radiation-induced base damage and (2) the direct induction of DNA strand breaks due to rupture at the phosphodiester bonds. Currently, cells are being isolated which are variant in these types of DNA repair.

Primary skin fibroblasts have been cultured from a patient who exhibited unusually severe skin reaction during radiation therapy. The cells showed increased radiosensitivity in vitro as compared to skin fibroblasts derived from normal individuals. A defective step in the DNA repair process has been demonstrated in these cells, which apparently is the first instance whereby correlation between in vivo and in vitro X-ray sensitivity with a specific molecular process has been successful. Clinical radiosensitivity and/or chemical sensitivity may, therefore, be useful in devising new systems for the study of DNA repair mechanisms.

It has been suggested that the phenomenon of finite in vitro life span ($\pm$ 50 generations) consistently observed in human diploid cells in culture may be a model for aging in the whole animal. It may also be a model for induced premature aging. Various hypothesis have been offered to account for the events leading to senescence in vitro, including accumulation of genetic damage in the genome and/or saturation of and "switching off" of repair mechanisms responsible for repairing lesions in DNA. Thus investigations were carried out on the ability of diploid cells to repair X-ray-induced single strand breaks as a function of the in vitro life span of the cells. Repair was studied also in cells from a patient with the Hutchinson-Gilford progeria syndrome of premature aging. These cells show a markedly reduced life span in vitro.

The skin fibroblasts biopsied from the progeria patient failed to show evidence of normal DNA strand rejoining after irradiation with 10 krads. The results suggest that the enzyme involved in DNA repair may be absent or greatly reduced in efficiency in cells of patients with this syndrome. Such a repair deficient cell strain may prove useful in the study of the molecular events associated with X-ray and chemical damage and its repair.

Preliminary studies with normal cell strains also indicate that normal diploid cells show a progressive decline in the efficiency of strand rejoining as they approach senescence. This may account in part for the increased incidence of some forms of cancer with aging. This phenomenon is currently under intensive investigation.

Reproductive Effects

Not surprisingly perhaps, in view of the variety of environmental agents under investigation, continuing studies show a wide range of reproductive changes and specificities upon exposure of different organism to toxic agents. Dr. Donald Sutherland at Rutgers University has shown that treatment of mosquito larvae with DDT, dieldrin, or trichlorfon results in an increase in basal follicles in the adult female. However, the increase in female reproductive tissue was not translated into an increase in fecundity as represented by egg deposition. These results reinforce previous findings by numerous investigators of an effect of some chlorinated hydrocarbon on the reproductive system, although neither these effects nor the quantitative relationships have been extended as yet to man. Thus, at present they remain only suggestive of possible effects on human reproduction, but they point up the need for further,more direct studies in human populations.

Work reported last year by Dr. Thomas Cade at Cornell University on chromosomal breaks caused by Aroclor 1254 in the dove was extended. Additional data indicate that second generation chromosomal breaks are not the only cause of embryonic death but that a behavioral aberration in the female dove may also be a factor. By using artificial incubation, the hatching success of the eggs was increased, suggesting that attentiveness of the female to the hatching of the eggs may have been reduced. While these studies are not conclusive, they suggest a heretofore unrealized behavioral anomaly resulting from PCB exposure.

Dr. Lee Wattenberg has investigated the possible protective action of benzpyrene hydroxylase inducers against benzpyrene-induced carcinogenesis in the offspring. He found that betanaphthoflavone, administered to the pregnant mouse on the eighth, ninth, or tenth day of pregnancy gave a very significant reduction (80 percent) in pulmonary adenomas in the offspring of the mother after subcutaneous administration of benzypyrene on day eleven, thirteen, or fifteen. 2-(4')-Iodophenylbenzothiazole, another known hydroxylase inducer, had little or no protective action. These studies indicate that the risk of cancer to the progeny of mothers at risk from benzypyrene exposure can be reduced by certain, but not all, inducers of this enzyme.

The perinatal effects of a number of pesticides were examined by Dr. James Gibson of Michigan State University. The pesticides studied were Bidrin (insecticide), paraquat (herbicide), and dinoseb (herbicide), and 2,4,-dinitrophenol. Bidrin was shown to be teratogenic in chickens but not in mice. AChE enzyme activity in the mouse was not changed. Dinoseb did not affect fetal resorption rate but produced some teratogenisis, involving gross soft tissue and skeletal anomalies. Paraquat administered to pregnant mice on gestational days eight through sixteen induced 85 percent mortality in dams by day fifteen. Embryotoxicity was seen only at doses that induced maternal mortality. Teratogenicity was also evident, consisting of a small increase in skeletal anomalies. Paraquat was partially restricted from access to the embryo so that levels did not reach those found in the mother.

Dr. Raymond Harbison of Vanderbilt University has found that treatment of pregnant mice with parathion produces a significant incidence of in utero

deaths of pups. Depression of fetal cholinesterase levels correlated directly with the incidence of *in utero* deaths. A depression of 20 percent in the enzyme resulted in 90 percent fetal death. Phenobarbital pretreatment was effective in preventing the effect. The protective action apparently results from alteration of placental transfer of paraoxon rather than on metabolism of parathion, since induction of increased activity of oxidases leads to toxicity enhancement of parathion via conversion to paraoxon.

Dr. Harbison found that DDT, chlordane, and heptachlor were less toxic to the newborn before treatment with phenobarbitol. Thus, induction of mixed function oxidases in the newborn apparently has a detrimental effect in case of these pesticides.

In work with lead, Dr. Harbison showed that lead accumulates to higher levels in the fetus and newborn than in the mother, with significantly higher levels in the brain. The increased frequency of lead encephalopathy in the newborn thus may be attributable to a preferential distribution to the CNS of the fetus or newborn.

Dr. Virgil Ferm of Dartmouth College has investigated the biological enigma of copper in embryogenesis. On the one hand, there is evidence that copper deficiency is detrimental to embryonic survival in the rat and chicken. On the other, there is evidence that an excess of copper has a detrimental effect on implantation and blastocyst development. In an effort to define more precisely the action and role of copper in early embryonic development, Dr. Ferm treated hamsters on the eighth day of gestation with chelated and nonchelated copper compounds. He found an increase in the number of embryonic resorptions as well as the appearance of developmental malformations. Malformations of the heart appeared to be a specific result of copper. Copper in the chelated form of copper citrate was more embryopathic than that administered as copper sulfate.

In the same laboratory, Dr. Thomas Gale has explored the teratogenic effects of mercurials, lead, chromium, and ytterbium. With inorganic mercury, he found the major manifestation of embryonic damage included an increased resorption rate as well as an increased frequency of small, retarded and edematous embryos. Several other minor malformations were noted. Comparative studies on the teratogenicity of chromium and lead show that the metals have similar effects and that mixtures of the two are additive with respect to the degree of edema, embryo growth retardation, and tail abnormalities.

Two grantees, Dr. Modesto Yang of Michigan State and Dr. George Okita of Northwestern University, have been concerned with teratogenicity of methyl mercury. Dr. Yang found that feeding of the compound to rats on the tenth day of pregnancy produces a number of congenital disorders in the young, including hemorrhage in the cerebrum and hind legs, brains with diffuse structure, and abnormal proportion between the head and body.

Dr. Okita's work is concerned with the possible role of brain Na, K-ATPase inhibition by mercurials as a controlling factor in methyl mercury effects. The experiments indicate that MeHg administered as a single dose on the tenth or eleventh day of gestation induces embryotoxic effects and changes in

behavioral patterns of the newborn. These effects are correlated with decreased fetal brain Na, K-ATPase. However, while the correlation is suggestive, the cause-effect relations cannot yet be considered conclusive.

Dr. Raymond Harbison at Vanderbilt has studied the developmental toxicology and teratogenicity of a number of pesticides in the rat. Two groups of pesticides were studied in adult and newborn animals. The chlorinated pesticides heptachlor, chlordane, and DDT are less toxic in the newborn when compared to the adult, but phenobarbital pretreatment potentiates their toxicity in the newborn. Parathion, on the other hand, is more toxic in the newborn when compared to adult, but phenobarbital pretreatment antagonizes the toxicity. Increased sensitivity of the newborn to parathion toxicity appears to be a result of decreased brain cholinesterase and higher plasma and brain levels of paraoxon, the active cholinesterase inhibitor, as well as increased in situ production of paraoxon in brain tissue. These data suggest that chlorinated pesticides are generally less toxic in the newborn and might be less hazardous for use in areas where young children would be exposed. Parathion is more toxic in the newborn and probably represents a greater hazard for use in areas where children would be exposed.

Dr. Harbison also showed that significant changes in the placental transfer of diphenylhydantoin (DPH) occur in relation to gestational age. Administration of ^{14}C-labeled DPH to pregnant mice, rats, and hamsters resulted in significantly higher concentrations of DPH in the fetal tissue during early and late gestation near the time of parturition, while low levels were present during the period of fetal morphogenesis. Concentrations of DPH in fetal and placental tissues also varied between the rat, mouse, rabbit, and hamster when compared at similar developmental stages, with the highest concentration being found in the mouse and the lowest in the rat. There was no apparent correlation with maternal plasma levels in these species. Significant extraplacental transfer of DPH to fetal and placental tissues and amniotic fluid has been shown to occur in the ligated rat uterus after intraperitoneal administration of ^{14}C-DPH. Thus placental transfer of DPH can be significantly affected by species, gestational age, and route of administration.

Mycotoxins. Where the principal dietary intake is grain, pregnant women are confronted with the problem of ingesting moldy corn, oats, rye, and rice. Moldy grains may significantly affect the developing embryo and fetus, as shown by workers at Vanderbilt University. Administration of rubratoxin B to pregnant mice at different stages of pregnancy produced various nonspecific anomalies, including brain, eye, ear, facial, lung, liver, and kidney abnormalities, which have not been previously reported. A dose-response relationship is seen by an increase in both in utero mortality and reduction of body weight of surviving fetuses with increasing dosage. None of the pregnant mothers that received a total of 1.0 mg/kg on one day or 1.5 mg/kg on three consecutive days of pregnancy exhibited any signs of toxicity or died following treatment. Administration of high doses of the toxin, however, produced maternal body weight loss in pregnant mice, particularly at advanced stages of gestation. This loss in body weight was attributed to the high incidence of fetal resorptions. The incidence of feto-toxicity resulting from rubratoxin B treatment may be explained in part by concentration at site of action, since the incidence of anomalies is dose related.

Chlorinated hydrocarbons. The effect of chlorinated hydrocarbons on reproduction has also been studied by Dr. Virgil Freed and his colleagues at Oregon State University. Experiments involving DDE and DDT show no significant effects on reproductive performance of male chickens as characterized by semen volume, fertility, and hatchability of eggs. Body and testes weights appeared more affected by DDT than DDE treatment. In both trials, characteristic tremors associated with DDT toxicity were noted at 31 weeks of age on one male on DDE and at 15 weeks of age in another male on DDT. Only when there was a reduction in testes and body weights did semen volume appear to be affected.

A recent report indicated that technical grade DDT added to the diet of White Leghorn layers produced an adverse effect on egg shell thickness. Investigations by the Oregon workers have involved chemically pure p,p'-DDT. Accordingly, it was felt advisable to compare the effects of technical and pure DDT. In this experiment egg production was adversely affected with technical grade DDT but not by pure DDT, so that impurities rather than DDT per se may be the major factor in the observed results. However, this conclusion needs further verification in view of effects of DDT on egg production in birds reported by others.

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